



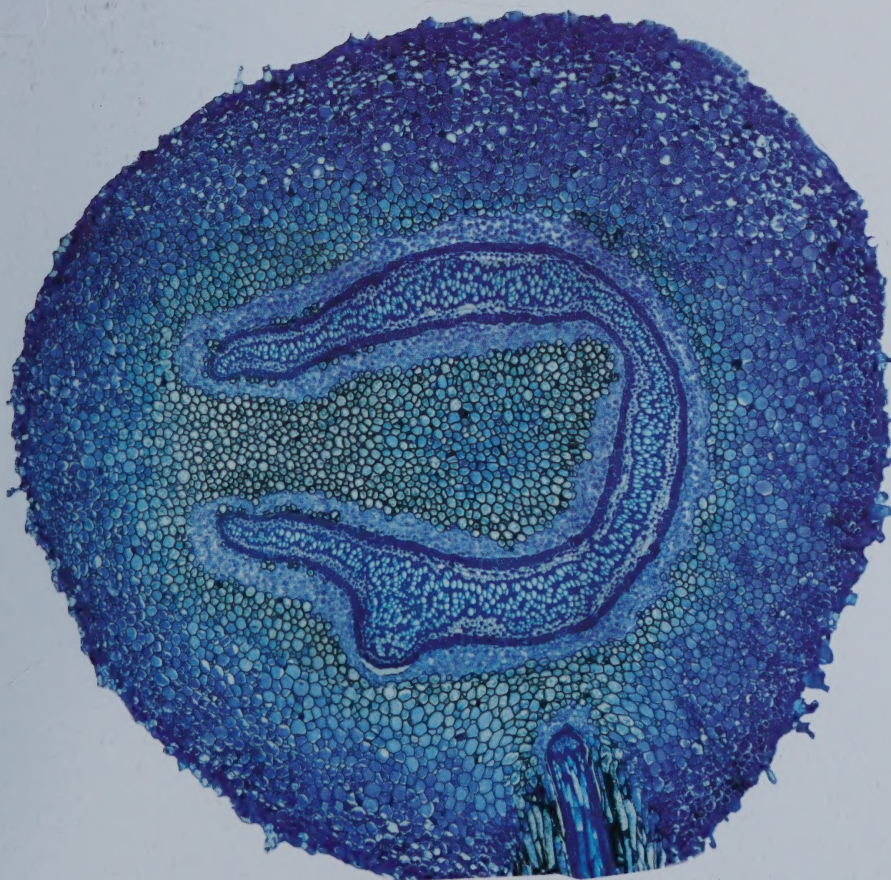
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Morpho-anatomical Studies and Evolutionary Interpretations of the Rhizomes of Extant Dennstaedtiaceae

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ABSTRACT.—Dennstaedtiaceae is a monophyletic, extant family of ferns with sub-cosmopolitan distribution and ca. 265 species. Rhizome morphology in the Dennstaedtiaceae is relatively well known, yet its anatomy has been poorly explored. We performed morpho-anatomical studies on 26 taxa from eight genera, mostly from Brazil, Japan, and New Zealand. Among the studied species, the main rhizome type is long-creeping with alternate phyllotaxy and a solenostele *s. str.* The exception is *Blotiella lindeniana*, which has ascending rhizomes with radial phyllotaxy and a dictyostele. A polycyclic solenostele was found in *Dennstaedtia* (“*Patania*”) *cornuta*, *D.* (“*Patania*”) *dissecta*, and two subspecies of *Pteridium arachnoideum*. Lateral-line aerenchyma characterized *Blotiella lindeniana*, *Histiopteris incisa*, *Hypolepis* spp., *Paesia* spp., and *Pteridium arachnoideum* subsp. The position of sclerified parenchyma in the cortex and pith varied among species. Our data enabled us to hypothesize evolutionary patterns of rhizome evolution in extant lineages of the Dennstaedtiaceae. The “Monachosoridaeae” has retained ancestral conditions: short-creeping to ascending rhizomes with dictyostele *s. str.* In the “Hypolepidoideae”, two notable changes occurred in *Blotiella* and *Pteridium* (in which the solenostele evolved into a polycyclic solenostele). In the “Dennstaedtioideae”, one notable change occurred in *Dennstaedtia* (“*Patania*”), namely parallel evolution with *Pteridium* (in which the polycyclic solenostele has also evolved). We present illustrations, morpho-anatomical descriptions, evolutionary inferences based on the available phylogeny, and supporting tools for studying the taxonomy, systematics, phylogeny, and paleontology of the group.

KEY WORDS.—ancient ferns, bracken fern, amphiphloic siphonostele, solenostele

Dennstaedtiaceae Lotsy is a monophyletic family of extant leptosporangiate ferns containing 10 genera and ca. 265 living species (PPG I, 2016). The family has a subcosmopolitan distribution, primarily due to the widely-distributed bracken ferns, *Pteridium* Gled ex Scop. (Der *et al.*, 2009; Tryon, 1941).

Although the Cretaceous diversification of the Polypodiales (*sensu* PPG I, 2016) has been controversial, Schneider, Schmidt, and Heinrichs (2016) just recently described a leaf from the mid-Cretaceous, *Krameropteris resinatus* H.Schneid *et al.*, with an undeniable cathetogyrate sporangium, and the authors ascribed it to the Dennstaedtiaceae. This discovery supports the molecular clock estimates for the origin of the family, of ca. 115 mya (Schneider *et al.*, 2004). Three other fossil genera are also ascribed to the

family: *Microlepiopsis* Sebert & Rothwell, also from the Cretaceous; *Dennastra* McIver & Basinger; and *Dennstaedtiopsis* Arnold & Daugherty (Collinson, 2001; Fossilworks, 2016; Sebert and Rothwell, 2003; Shi, Schopf, and Kudryavtsev, 2013).

As currently defined (Smith *et al.*, 2008; PPG I, 2016), most extant genera of Dennstaedtiaceae are probably monophyletic, except possibly for *Hypolepis* Bernh. (Brownsey, 1983) and for the clearly paraphyletic *Dennstaedtia* Bernh. (Perrie, Shepherd, and Brownsey, 2015; Schneider, Schmidt, and Heinrichs 2016; Schuettpelz and Pryer, 2007).

Stems in Dennstaedtiaceae are generally long-creeping rhizomes, and both their external morphology and internal anatomy are fairly well known (Bower, 1926; Gruber, 1981; Gwynne-Vaughan, 1903; Kaur, 1971; Keating, 1968; Kramer, 1990; Nair and Sem, 1974; Navarrete and Øllgaard, 2000; Ogura 1972; Schwartzburd and Prado, 2015; Troop and Mickel, 1968; Tryon and Tryon, 1982; Watt, 1940). However, research focusing on rhizome anatomy in the family has addressed only a few genera and species, and some features of the species, such as their lateral-line aerenchyma, have been poorly analyzed.

The anatomy of stems, especially of their vascular system, is considered to be a conserved character, as these organs are affected by only few environmental influences and have undergone few evolutionary modifications (Srivastava and Chandra, 2009). It has been hypothesized that the diversity of primary vascular systems was established early in the evolution of vascular plants (Beck, Schmid, and Rothwell, 1982). Studies on stem morpho-anatomy may yield important information on the relationships between extinct and extant taxa (Cantrill, 1997; Matsumoto *et al.*, 2006) as well as on the evolution of monophyletic lineages (Ebihara, 2007; Pryer, Smith, and Skog, 1995; Roux and Wyk, 2000; Smith, 1995).

In this paper, we provide an initial database on morphological features combined with anatomical characteristics of selected species from some extant Dennstaedtiaceae genera, in order to contribute complementary morphological data for circumscription of genera and to infer the evolutionary history of rhizomes in the family.

MATERIAL AND METHODS

We sampled 26 taxa (23 species, two subspecies, and one variety) belonging to eight genera of the Dennstaedtiaceae (*sensu* PPG I, 2016), mostly from Brazil, Japan, and New Zealand (Appendix 1). Sixty two specimens were analyzed, of which 35 were freshly collected and 27 were obtained from exsiccates (from herbaria CRH, TNS, and VIC). Rhizome external morphology was analyzed by naked eye and under a stereo microscope (model SMZ 068, Motic, China).

For the anatomical analysis, fragments were sampled from the internode mid regions of freshly collected rhizomes, fixed in FAA₅₀ (Johansen, 1940), dehydrated in an ethanol series, and embedded in methacrylate resin (Leica Historesin, Germany). Transversal and longitudinal sections 5–7 μm thick were obtained using an advance rotary microtome (model Spencer 820,

American Optical Corporation, USA). Sections were stained with toluidine blue, pH 7.2 (O'Brian and McCully, 1981), and mounted in synthetic resin (Permunt-Fischer). For samples from herbarium materials, rhizome fragments were first rehydrated (Smith and Smith, 1942) and then subjected to the method described above. We also performed a Phloroglucinol-HCl test for lignin (Johansen, 1940).

Tracheal elements were isolated through maceration and then identified. Rhizome fragments were fixed in Jeffrey solution (Johansen, 1940) for ca. 20 h, washed with distilled water and 50% ethanol, and stained with 1% safranin in 50% ethanol for five h. Samples were then washed in 30% ethanol and mounted on temporary microscopic glass slides. Samples were documented and photographed using a photomicroscope (model AX70TRF, Olympus Optical, Japan) coupled with a digital camera (AxioCam HR3, Zeiss, Germany).

Classification of the vascular system followed Schmid (1982). Our evolutionary interpretations were based on the available phylogeny of the Dennstaedtiaceae, which was constructed using *rbcL* sequences (Perrie, Shepherd, and Brownsey, 2015; and Schneider, Schmidt, and Heinrichs, 2016), excluding *Saccoloma* species from the former study. Sequences of *Saccoloma* spp. (analyzed by Wolf, 1995), which nested within Dennstaedtiaceae, were later found to be contaminated (Wolf, pers. comm.; see also PPG I, 2016). In the phylograms of Perrie, Shepherd, and Brownsey (2015) and Schneider Schmidt, and Heinrichs (2016), we inserted the species we analyzed, which did not have available *rbcL* sequences in GenBank (Fig. 1). Our insertions, while speculative (dotted lines), were based on morphological criteria and unpublished *trnL-trnF* sequences of *Hypolepis rugosula* subsp. *pradoana* Schwartsb. and *H. stolonifera* Fée var. *stolonifera*, which were compared with other *Hypolepis* spp. (Schwartzburd and Perrie, *unpubl. data*). To avoid bias, we inserted most species in polytomies. A dual hypothesis was elaborated for *Dennstaedtia globulifera* (Poir.) Hieron. (indicated by an asterisk).

We named the three main clades of Dennstaedtiaceae (Perrie, Shepherd, and Brownsey, 2015) as: "Monachosoroideae" (containing only *Monachosorum* Kunze), "Hypolepidoideae" (cont. *Paesia* A.St.-Hil., *Histiopteris* J.Agardh, *Blotiella* R.M.Tryon, *Hypolepis* Bernh., and *Pteridium*), and "Dennstaedtioideae" (cont. paraphyletic *Dennstaedtia* Bernh., *Oenotrichia* Copel., *Leptolepia* Kuhn, and *Microlepia* C.Presl). We also put alternative generic names for some *Dennstaedtia* species in brackets, similar to Schneider, Schmidt, and Heinrichs (2016). One sensitive difference from Schneider, Schmidt, and Heinrichs (2016) regards the placement of "*Patania*". The type of *Patania* C.Presl is *P. obtusifolia* (= *D. obtusifolia*), which forms a species complex with *D. dissecta* (Sw.) T. Moore and *D. cornuta* (Kaulf.) Mett. Thus, we named this clade "*Patania*"; and not *D. scabra*, as Schneider, Schmidt, and Heinrichs (2016) did. We polarized our tree using some basal Pteridaceae genera, such as *Llavea* Lag. and *Cryptogramma* R. Br. (e.g., Pabón-Mora and González, 2016). That baseline information was used to describe the rhizome of our hypothetical ancestor of the Dennstaedtiaceae.

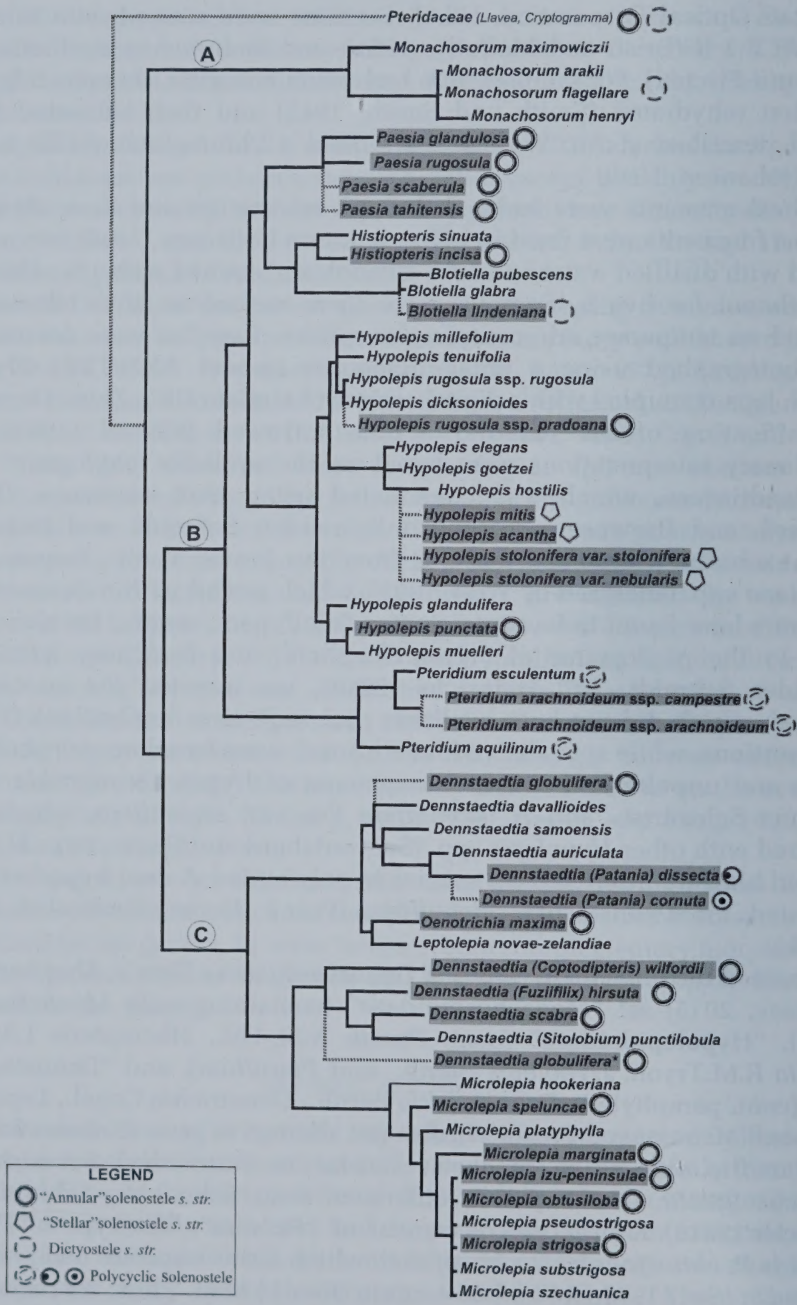


FIG. 1. Phylogram of Dennstaedtiaceae, according to Perrie, Shepherd, and Brownsey (2015) and Schneider, Schmidt, and Heinrichs (2016). Species sampled in our study are highlighted in gray. Dotted lines show tentative positioning of species not sampled in the original phylogenies. Asterisks indicate alternate hypotheses of the relationships in *Dennstaedtia globulifera*.

RESULTS

Rhizome morphology is relatively homogeneous among the studied species. Among the species studied, the main rhizome type was the creeping rhizome (Fig. 2C, 2D, 3A, 3D, 3F, 4A, 4B, 4D, 4F, 4G) with alternate phyllotaxy (e.g., Fig. 4G), except in *Blotiella lindeniana* (Fig. 2A), which has ascending rhizomes with radiate phyllotaxy. With a few exceptions of either glabrous or glabrescent rhizomes (*Dennstaedtia* (Patania) *cornuta*), most species are furnished with catenate hairs (Figs. 3E, 4C, E, 7H). *Histiopteris incisa* has also comose proto-scales (Figs. 3B, 7G). Epipetiolar buds (e.g., Fig. 3F), secondary roots (e.g., Fig. 3F), and catenate hairs are found at the base of petioles in most species.

All species have an uniseriate epidermis. The lateral-line aerenchyma is present in *Blotiella lindeniana* (Figs. 2B, 8C, E), *Histiopteris incisa* (Figs. 3A, C), *Hypolepis* spp. (Figs. 3G, 8D, F), *Paesia* spp. (Figs. 3E, 8B), and *Pteridium arachnoideum* subsp. (Figs. 2E - arrows, 8A). Lines of aerenchyma run laterally on rhizomes and have stomata on their surface (Figs. 8D, E). The aerenchyma below the epidermis is characterized by conspicuous intercellular gaps and by intercellular protuberances (IP) of cell walls (Fig. 8F). *Blotiella lindeniana*, *Paesia* spp., and *Pteridium arachnoideum* subsp. have "neck-like" extensions from the inner cortex to the aerenchyma (Figs. 8A-C). Neither *Histiopteris incisa* nor *Hypolepis* spp. have such "neck-like" extensions (e.g., Fig. 8D).

The location of sclerified parenchyma layers in the cortex varies among species. This tissue is composed by slightly longer cells with thick cell walls, but without lignin deposit (thus not composing a true sclerenchyma). Those layers may be located in the outer, medium, and/or inner cortex. *Hypolepis* spp., *Histiopteris incisa*, and *Oenotrichia maxima* have sclerified parenchyma in the inner cortex (next to the endodermis) (e.g., Fig. 7E). On the other hand, the inner cortex of *Blotiella lindeniana*, *Paesia* spp., *Dennstaedtia* spp., *Microlepia* spp., and *Pteridium arachnoideum* subsp. has unmodified parenchyma (e.g., Fig. 7F).

The vascular systems of all studied species are classified into the major group of amphiphloic siphonostele *sensu* Schmid (1982). In this group, steles are enclosed by both an inner and outer endodermis. In all species we studied, the endodermis is formed by tabular cells without lignin deposit. The pericycle below it is formed by two layers of isodiametric cells (e.g., Fig. 7A). We found three types of steles (*sensu* Schmid 1982): 1. solenostele *s. str.*, 2. polycyclic solenostele, and 3. dictyostele *s. str.*

Solenostele *s. str.* is, by far, the most common stele type in the Dennstaedtiaceae (Figs. 5A-C, E, 6C-H). This stele type was found in *Dennstaedtia globulifera* (Fig. 6D), *D. (Fuziifilix) hirsuta* (Fig. 6E), *D. scabra*, *D. (Coptopteris) wilfordii* (Fig. 6C), *Histiopteris incisa* (Fig. 5C), *Hypolepis acantha* (Fig. 5A), *Hyp. mitis*, *Hyp. punctata* (Fig. 5B), *Hyp. rugosula* subsp. *pradoana*, *Hyp. stolonifera* var. *stolonifera*, *Hyp. stolonifera* var. *nebularis*, *Microlepia izu-peninsulae*, *M. marginata*, *M. obtusiloba* (Fig. 6G), *M.*

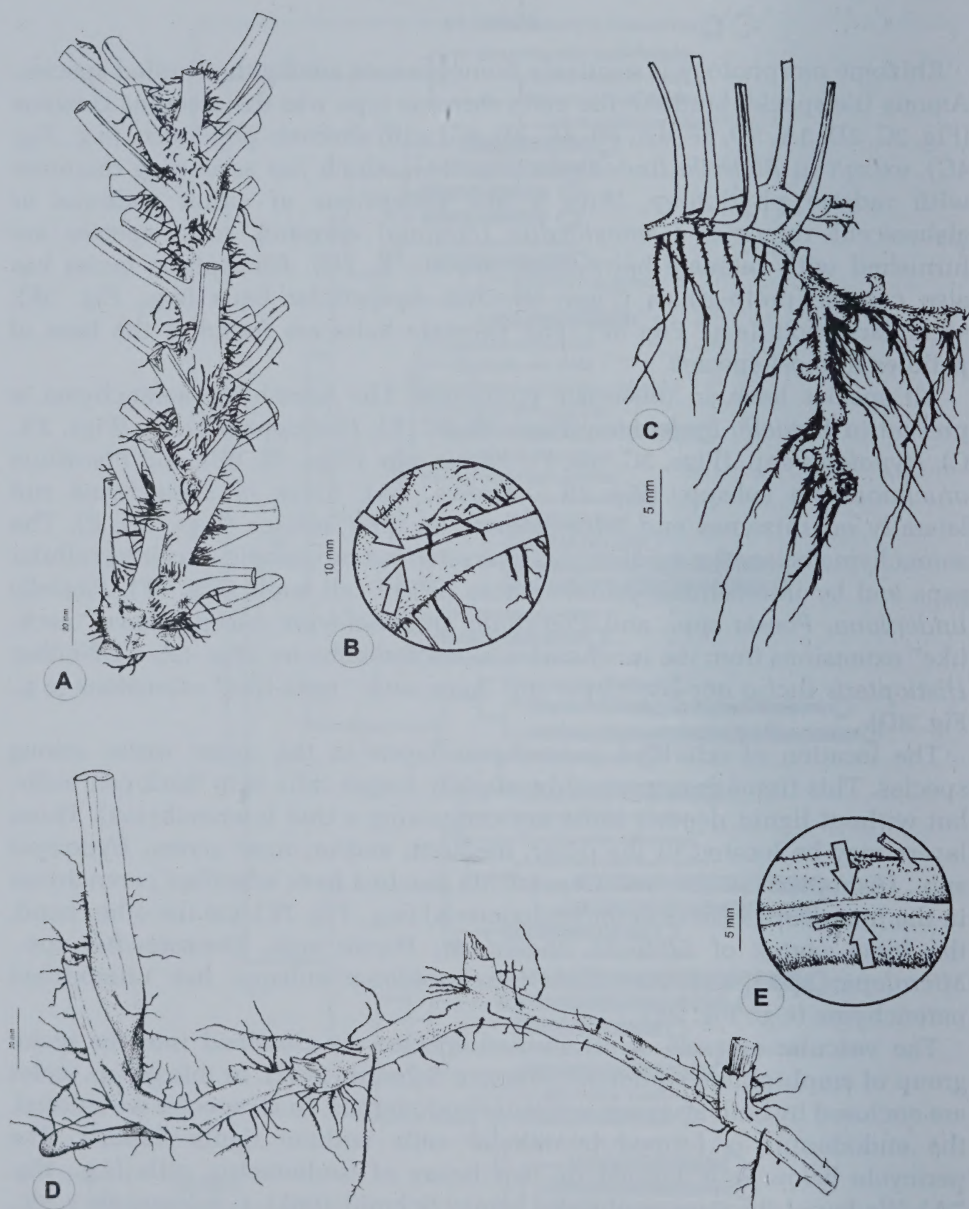


FIG. 2. Habit and rhizome morphology in the Dennstaedtiaceae. A, B. *Blotiella lindeniana*. A. Ascending rhizome. B. Arrows indicate the lateral-line aerenchyma, which extends from the rhizome to the petiole. C. Creeping rhizome of *Dennstaedtia (Coptodipteris) wilfordii*. D, E. *Pteridium arachnoideum* subsp. *campestre*. D. Creeping rhizome. E. Arrows indicate the lateral-line aerenchyma.

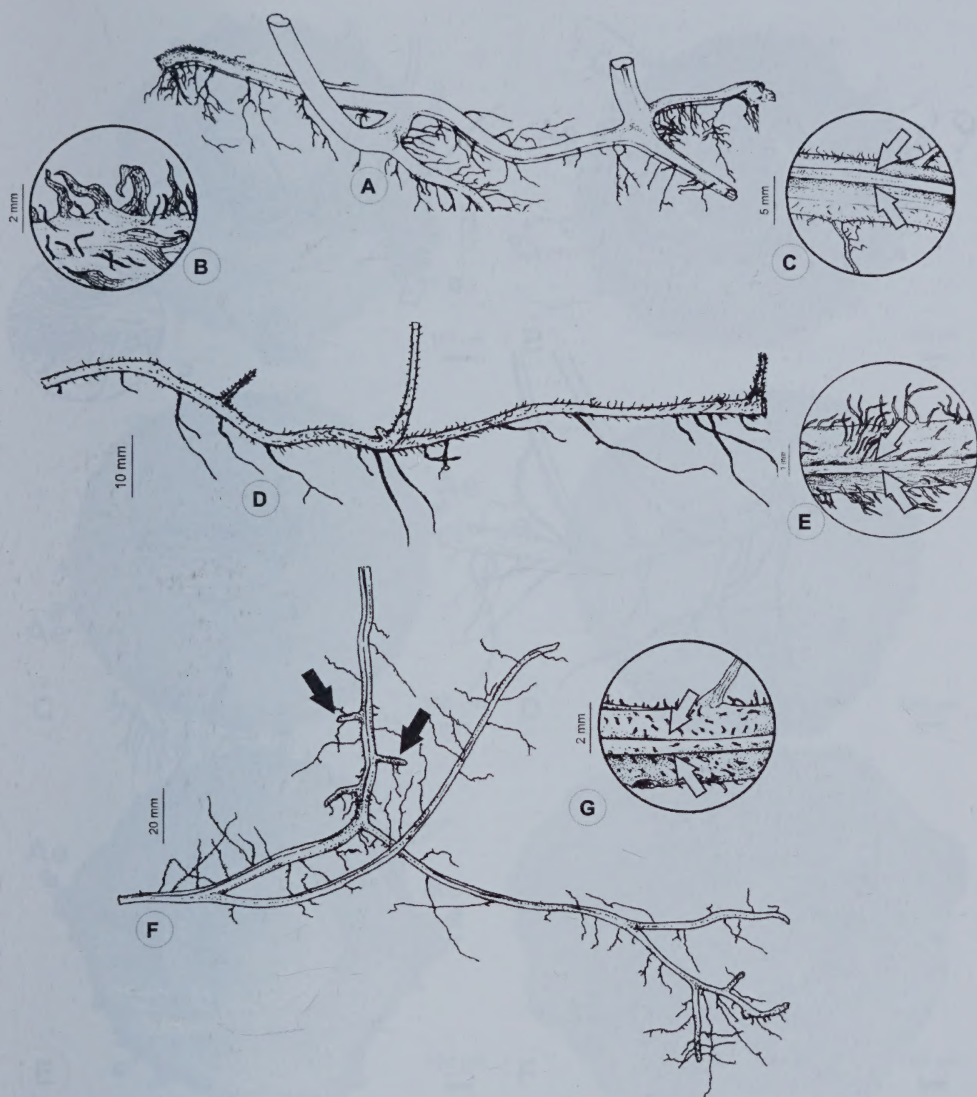


FIG. 3. Habit and rhizome morphology in the Dennstaedtiaceae. A–C. *Histiopteris incisa*. A. Creeping rhizome. B. Detail of comose proto-scales and hairs. C. Arrows indicate the lateral-line aerenchyma. D, E. *Paesia glandulosa*. D. Creeping rhizome. E. Arrows indicate the lateral-line aerenchyma. F, G. *Hypolepis stolonifera* var. *nebularis*. F. Creeping rhizome (Arrows indicate epipetiolar buds). G. Arrows indicate the lateral-line aerenchyma.

speluncae (Fig. 6H), *M. strigosa*, *Oenotrichia maxima* (Fig. 6F), *Paesia glandulosa* (Fig. 5E), *P. rugosula*, *P. scaberula*, and *P. tahitensis*. All these species have annular solenosteles, except for *Hyp. acantha*, *Hyp. mitis*, *Hyp. stolonifera* var. *stolonifera*, and *Hyp. stolonifera* var. *nebularis*, which have stellar solenosteles with well-defined protoxylem poles (e.g., Fig. 5A).

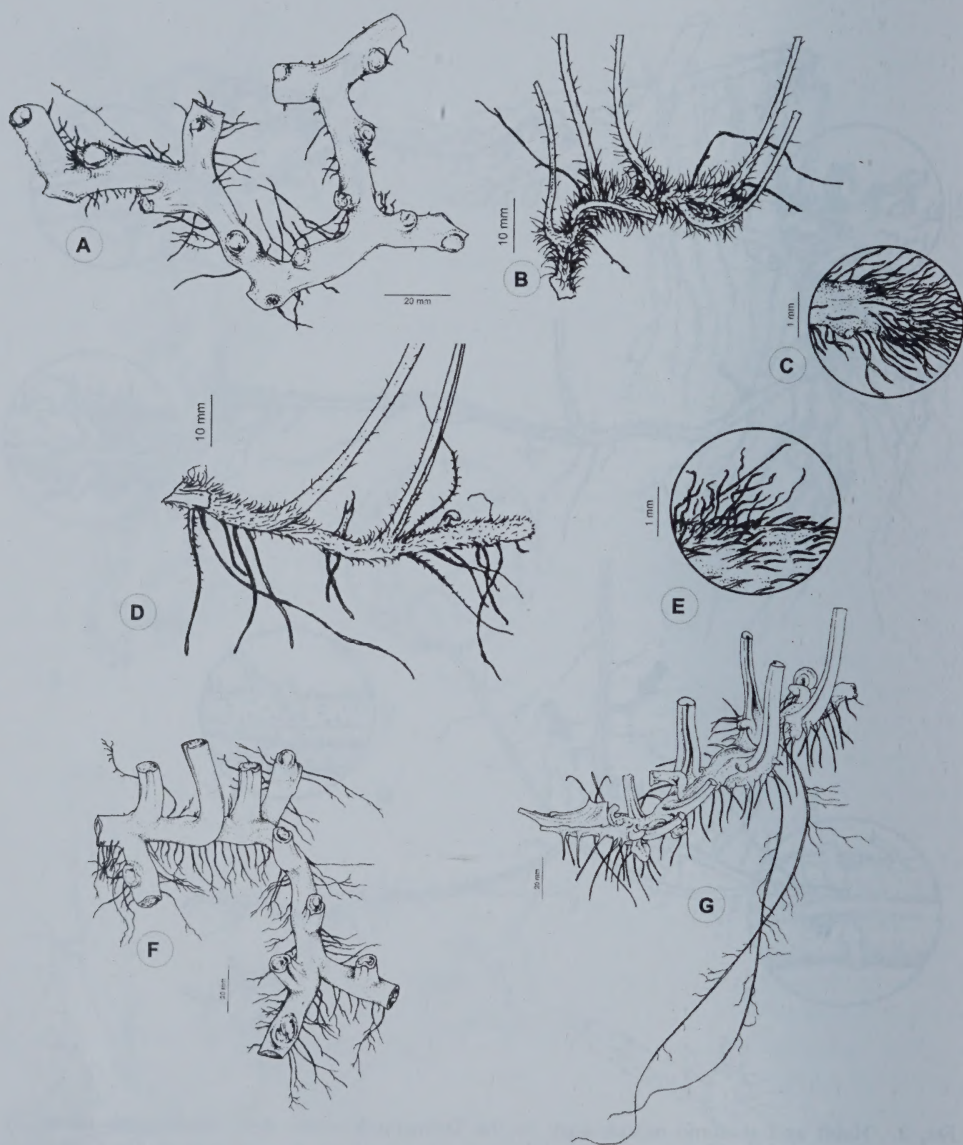


FIG. 4. Habit and rhizome morphology in the Dennstaedtiaceae. A. Creeping rhizome in *Dennstaedtia globulifera*. B, C. *Dennstaedtia* (*Fuziifilix*) *hirsuta*. B. Creeping rhizome. C. Detail of catenate hairs. D, E. *Microlepia marginata*. D. Creeping rhizome. E. Detail of catenate hairs. F. Creeping rhizome in *Microlepia speluncae*. G. Short-creeping rhizome in *Dennstaedtia* (*Patania*) *cornuta*.

The two other stele types are rare in the family. *Blotiella lindeniana* has a dictyostele s. str. (Fig. 5D), while *Dennstaedtia* ("Patania") *dissecta*, *D.* ("Patania") *cornuta*, *Pteridium arachnoideum* subsp. *arachnoideum*, and *P. arachnoideum* subsp. *campestre* have a polycyclic solenostele (Figs. 5F, 6A–

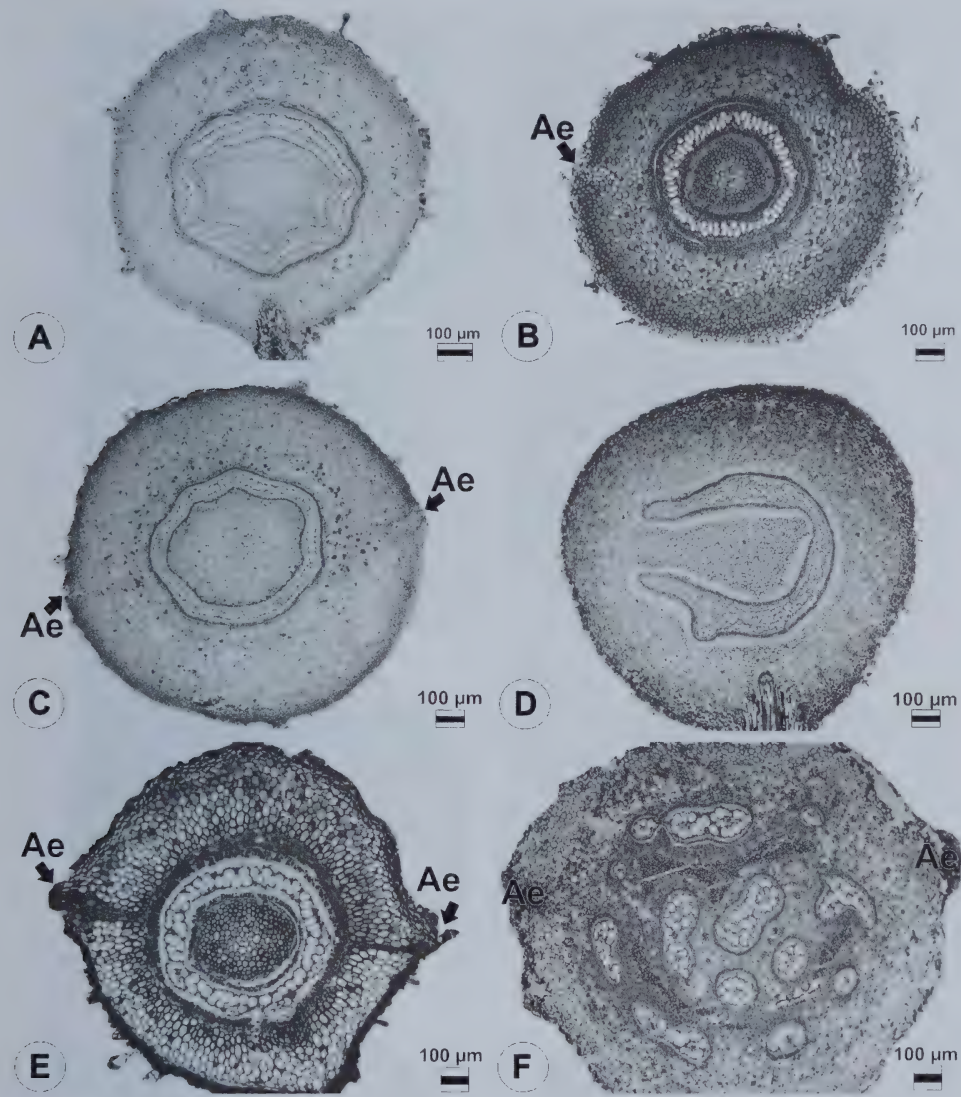


FIG. 5. Anatomical overview of rhizomes in the Dennstaedtiaceae (in cross section). A. *Hypolepis acantha*. B. *Hypolepis punctata*. C. *Histiopteris incisa*. D. *Blotiella lindeniana*. E. *Paesia glandulosa*. F. *Pteridium arachnoideum* subsp. *campestre*. "Ae" = lateral-line aerenchyma.

B). The polycyclic solenostele differed among these four taxa. In *D. dissecta*, there is a vascular protuberance inward the annular solenostele (Fig. 6B). In *D. cornuta*, there are either two concentric annular solenosteles (Fig. 6A), or occasionally the inner solenostele may have no pith, thus resembling a protostele. In *Pteridium arachnoideum* subsp., there are two concentric rows of meristeles (Figs. 5F, 7B).

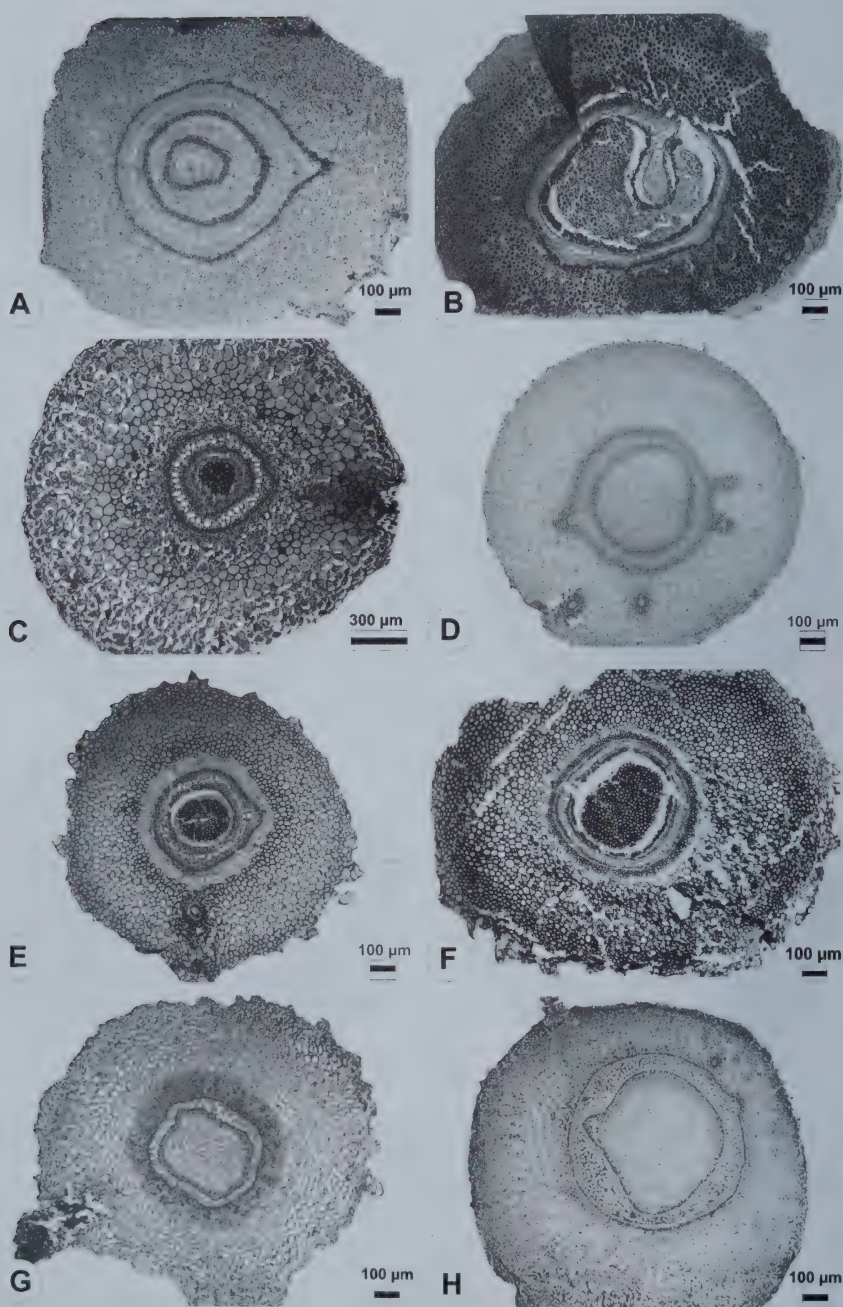


FIG. 6. Anatomical overview of rhizomes in the Dennstaedtiaceae (in cross section). A. *Dennstaedtia* (Patania) *cornuta*. B. *Dennstaedtia* (Patania) *dissecta*. C. *Dennstaedtia* (Coptodipteris) *wilfordii*. D. *Dennstaedtia* *globulifera*. E. *Dennstaedtia* (Fuziifilix) *hirsuta*. F. *Oenotrichia* *maxima*. G. *Microlepia* *obtusiloba*. H. *Micropelia* *speluncae*.

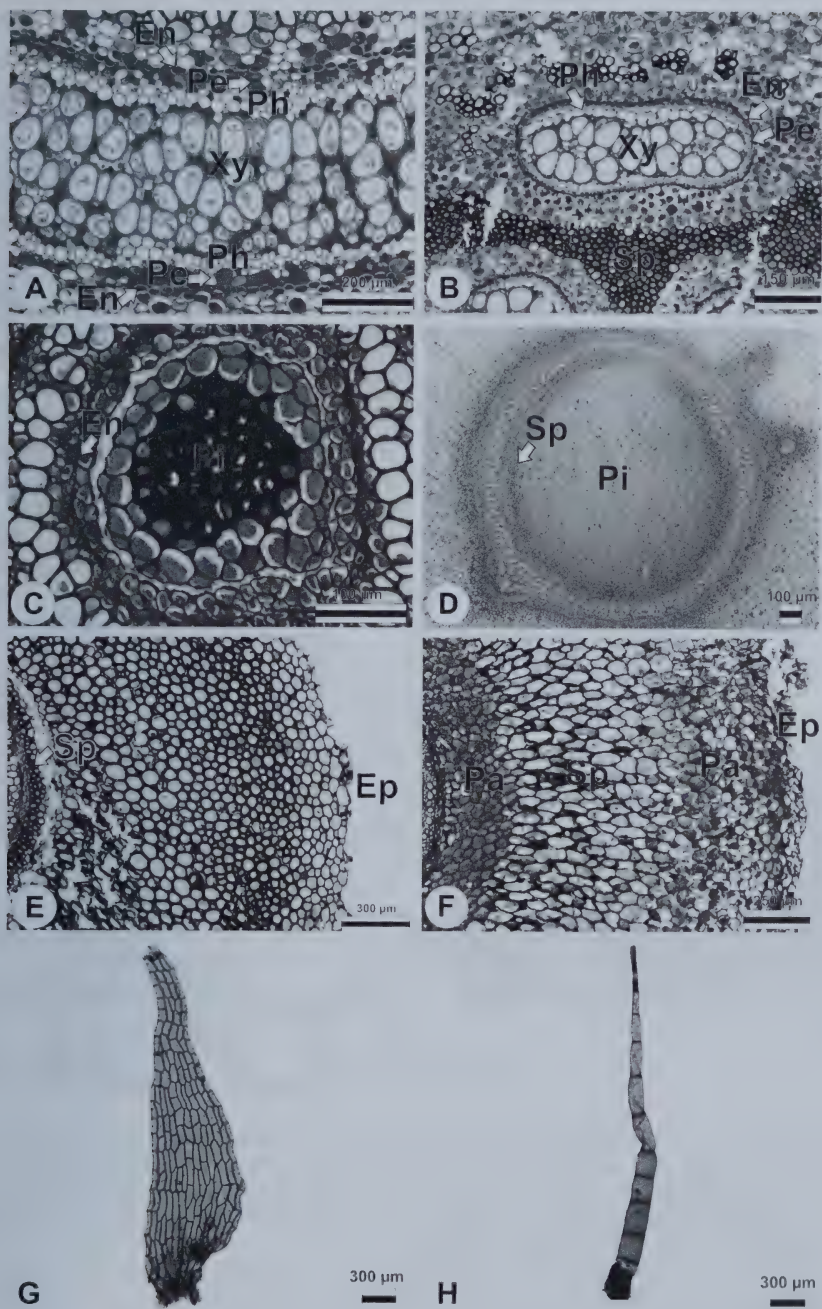


FIG. 7. Anatomical details of rhizomes in the Dennstaedtiaceae (A–F in cross section). A. Vascular bundle with inner and outer endodermis in *Microlepia speluncae*. B. Meristele of *Pteridium arachnoideum* subsp. *campestre*. C. Inner pith with sclerified parenchyma in *Dennstaedtia (Coptodipteris) wilfordii*. D. Pith with only its outer layer having a sclerified parenchyma in *Dennstaedtia globulifera*. E. Cortex of *Oenotrichia maxima*. F. Cortex of *Microlepia marginata*. G. Comose proto-scales of *Histiopteris incisa*. H. Catenate hair of *Hypolepis acantha*. “En”= endodermis; “Ep”= epidermis; “Pa”= non-sclerified parenchyma; “Pe”= pericycle; “Ph”= phloem; “Pi”= pith; “Sp”= Sclerified parenchyma; Xy= xylem.

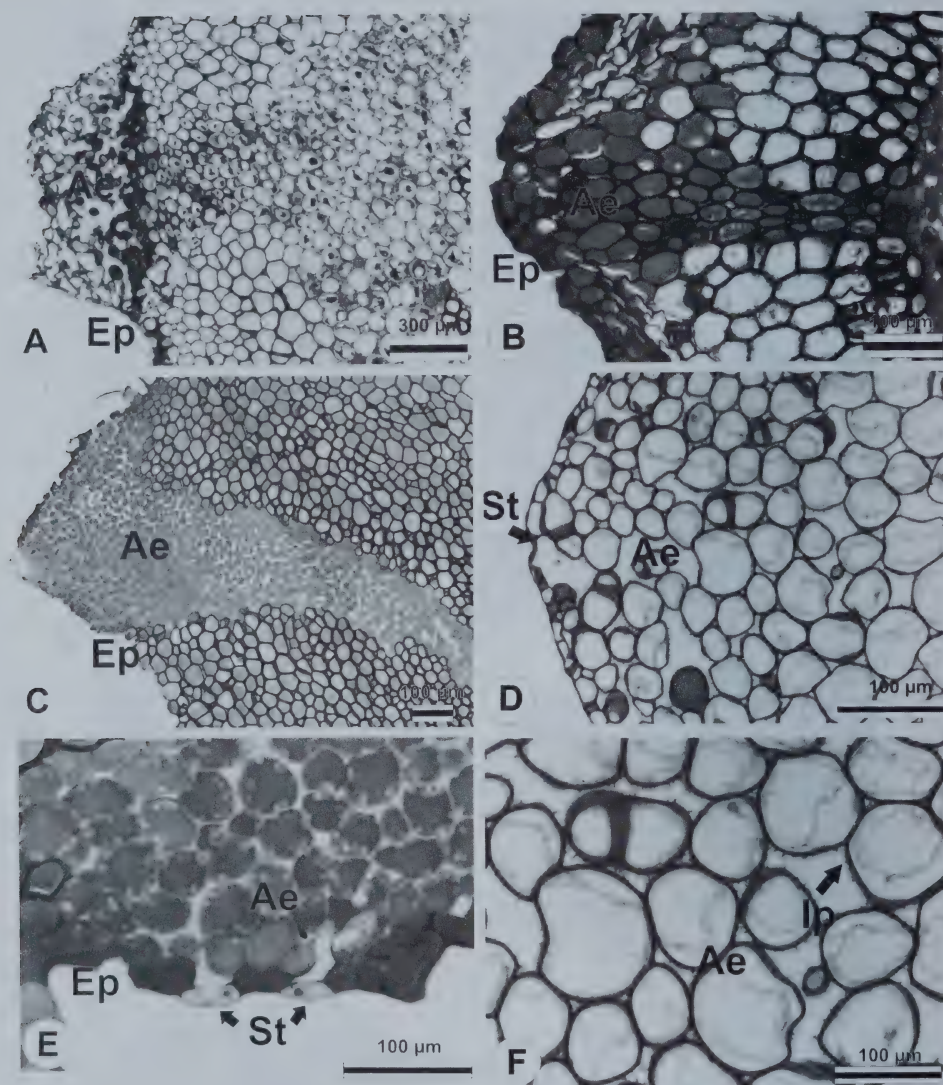


FIG. 8. Lateral-line aerenchyma in rhizomes of the Dennstaedtiaceae (in cross section). A. *Pteridium arachnoideum* subsp. *campestre*. B. *Paesia glandulosa*. C. *Blotiella lindeniana*. D. *Hypolepis acantha*. E. Detail of stomata on the epidermis of *Blotiella lindeniana*. F. Detail of inter-cellular protuberances in *Hypolepis acantha*. "Ae"= lateral-line aerenchyma; "Ep"= epidermis; "Ip"= inter-cellular protuberances; "St"= stomata.

All species have only scalariform tracheids as conducting cells in the xylem, except for *Pteridium arachnoideum* subsp., which also have vessel elements. Phloem in the species is formed by sieve cells.

Pith composition also varies among species. Some of them have sclerified parenchyma occupying almost the entire pith, while others have sclerified

parenchyma only in the outermost pith layers (i.e., those next to the endodermis). *Blotiella lindeniana*, *Dennstaedtia hirsuta*, *D. scabra*, *D. wilfordii*, *Microlepia marginata*, *M. izu-peninsulae*, *M. obtusiloba*, *M. strigosa*, *Oenotrichia maxima*, and *Paesia* spp. have sclerified parenchyma occupying almost the entire pith (e.g., Fig. 7C). On the other hand, in *D. globulifera*, *Histiopteris incisa*, *Hypolepis* spp., and *M. speluncae* only the outermost layers are occupied by sclerified parenchyma (e.g., Fig. 7D).

TENTATIVE KEY TO GENERA OF DENNSTAEDTIACEAE BASED ON THE MORPHO-ANATOMY OF THE STUDIED SPECIES (SOME DENNSTAEDTIA AND MICROLEPIA SPECIES CANNOT BE PRECISELY DISTINGUISHED):

- 1. Rhizomes solenostelic s. str.
- 2. Rhizomes bearing catenate hairs and comose scales *Histiopteris*
- 2. Rhizomes bearing catenate hairs only
- 3. Rhizomes with stellar solenostele s. str. *Hypolepis* (in part)
- 3. Rhizomes with annular solenostele s. str.
- 4. Lateral-line aerenchyma present
- 5. Pith mostly sclerified *Paesia*
- 5. Pith with only the peripheral region sclerified *Hypolepis* (in part)
- 4. Lateral-line aerenchyma absent
- 6. Inner cortex sclerified adjacent to the outer endodermis *Oenotrichia*
- 6. Inner cortex not sclerified
- 7. Pith with only the peripheral region sclerified
..... *Dennstaedtia* (*D. globulifera*) and *Microlepia* (*M. speluncae*)
- 7. Pith mostly sclerified
..... *Dennstaedtia* (*D. scabra*, *D. hirsuta*, *D. wilfordii*) and
Microlepia (*M. marginata*, *M. izu-peninsulae*, *M. obtusiloba*, *M. strigosa*)
- 1. Rhizomes with dictyostele s. str. or polycyclic solenostele
- 8. Rhizomes ascending, with dictyostele *Blotiella*
- 8. Rhizomes medium- to long-creeping, with polycyclic solenostele
- 9. Rhizomes long-creeping; polycyclic solenostele subdivided into meristeleles.
..... *Pteridium*
- 9. Rhizomes medium-creeping; polycyclic solenostele not subdivided into meristeleles *Dennstaedtia* (*Patania*)

DISCUSSION

Evolutionary interpretations.—Based on the outgroup (e.g., *Llavea*, *Cryptogramma*), we established a hypothesis on the rhizome of the family ancestor (Fig. 1). This hypothetical ancestor had a short-creeping to ascending rhizome, with dorsiventral symmetry, short internodes, either a solenostele s. str. or a transitional dictyostele s. str., and indumentum formed by scales. The first divergent lineage (“Monachosoridae”, Fig. 1 – clade A) from the ancestor retained its conserved features, which is similar to the basal Pteridaceae (Nair and Sem, 1974; Kramer, 1990). The exception is the indumentum, which consisted of hairs.

In the other lineage (a large clade containing “Hypolepidoideae” and “Dennstaedtioideae”, Fig 1 – clades B and C, respectively), a higher morpho-

anatomical diversification occurred in rhizomes. In this large clade, the main rhizome type is long-creeping with extended internodes, solenostele *s. str.*, and indumentum formed also by hairs.

Within the “Hypolepidoideae” (Fig. 1 – clade B), the long-creeping rhizome with solenostele *s. str.* is maintained in *Paesia* (Figs. 3D, 5E), *Histiopteris* (Figs. 3A, 5C), and *Hypolepis* (Figs. 3F, 5A, B), but two notable changes occurred within this clade. In *Blotiella*, the rhizome became truly ascending with radial symmetry, but also with a dictyostele *s. str.*, similar to the one of the ancestor (Figs. 2A, 5D). The other change occurred in *Pteridium*. The rhizome remained long-creeping, but the vascular system evolved into a polycyclic solenostele (Figs. 2D, 5F). The polycyclic solenostele of *Pteridium* is considered to be an advanced stage of the dictyostele *s. str.*, having probably derived from two concentric rings of dictyostele (Ogura, 1972). *Pteridium* has also vessel elements, which is a unique feature in the Dennstaedtiaceae. From the points of view of survival and colonizing success, the new features in *Pteridium* were extremely advantageous, in light of its current distribution and biomass, when compared with those of other Dennstaedtiaceae genera (e.g., Der *et al.*, 2009; Schwartsburd, Moraes, and Lopes-Mattos, 2014; Tryon, 1941).

The presence of comose proto-scales in rhizomes of *Histiopteris* (Figs. 3B, 7G) may be considered the result of reverse evolution. Within *Hypolepis*, there was also a slight change in shape of the solenostele *s. str.* Some species retained the annular solenostele (Fig. 5B), while others evolved the stellar solenostele with protoxylem poles (Fig. 5A).

In the “Dennstaedtioidae” clade (Fig. 1 – clade C), the long-creeping rhizomes with solenostele *s. str.* also remained as the main rhizome type, being present in *Microlepia* (Figs. 4D, F, 6G, H), *Oenotrichia* (Fig. 6F), and most species of *Dennstaedtia* (Figs. 4A, B, 6C–E), including “*Coptodipteris*” and “*Fuziifilix*”. Within “Dennstaedtioidae”, one notable change occurred. The rhizomes of *Dennstaedtia* “*Patania*” became medium-creeping, and the vascular system evolved from solenostele *s. str.* into a polycyclic solenostele (Figs. 4G, 6A, B). The polycyclic solenostele represents a parallel evolution between *Dennstaedtia* “*Patania*” (“Dennstaedtioidae” clade) and *Pteridium* (“Hypolepidoideae” clade). The shape of the polycyclic solenostele in *D. (Patania) dissecta* (Fig. 6B) is probably an intermediary condition between the solenostele *s. str.* and the shape found in *D. (Patania) cornuta* (Fig. 6A).

The three fossil genera with rhizomes currently ascribed to the Dennstaedtiaceae (*Dennastra*, *Dennstaedtiopsis*, and *Microlepiopsis*) are similar to the extant genera of the family, especially in their vascular system. This also indicates the conservation of the main characters along lineages of the Dennstaedtiaceae. The notable aerenchyma in *Dennstaedtiopsis aerenchymata* (Shi, Schopf, and Kudryavtsev, 2013) may either be only an isolated acclimatization to marshy places or represent a feature from another lineage of Dennstaedtiaceae that is now extinct.

Contributions to taxonomy and systematics.—Some variation in rhizomes of the Dennstaedtiaceae may be valuable to the taxonomy and systematics of the family. Differential distribution of the sclerified parenchyma in the pith and cortex (Figs. 7C–F) is an important feature to distinguish *Dennstaedtia s.l.*, *Hypolepis*, *Histiopteris*, and *Paesia*. This tissue type has taxonomic value in other fern families, and is responsible for protection and support of the stele (Ogura, 1972).

The lateral-line aerenchyma (Fig. 8) present in *Blotiella*, *Hypolepis*, *Histiopteris*, *Paesia*, and *Pteridium* also has taxonomic value. Such a lateral line has been described in many fern families, but primarily in petioles and not in rhizomes (Davies, 1991; Schwartsburd and Prado, 2015). Lateral-line aerenchyma has been reported in *Histiopteris incisa* and *P. aquilinum* (Ogura, 1972, as “pneumatic stripes”), and to a few species of *Dennstaedtia* and *Hypolepis* (Heidelberg, 1976). The occurrence of stomata and IP in the lateral-line aerenchyma has been reported by Barton, Overall, and Thomson (2015) in *P. aquilinum* subsp. *aquilinum*, *P. aquilinum* subsp. *wightianum*, and *P. esculentum* subsp. *esculentum*. On the other hand, IP had never been reported from any other Dennstaedtiaceae genus. These structures seem to be intimately related to the aerenchyma in the family.

Microlepia and *Dennstaedtia s.l.* (except “*Patania*”) have indistinguishable rhizome anatomies (Figs. 6C–E, G, H). *Dennstaedtia* was demonstrated to be paraphyletic by Perrie, Shepherd, and Brownsey (2015) and Schneider, Schmidt, and Heinrichs (2016), with most of its species nesting within *Microlepia* and other species nesting within another clade. *Dennstaedtia* will probably be split into two to several smaller genera so that a monophyletic classification may be established. Our anatomical data agree with the phylogenetic conclusions. Probably, “*Patania*” will be resurrected as a valid genus, and most diagnostic features will be based on rhizomes (medium-creeping, polycyclic solenostele, etc.).

Lastly, this work also aimed to provide the first database on rhizomes of the Dennstaedtiaceae. We hope our data will be useful for paleontologists to compare extinct and extant genera and help them classify fossil specimens into extant genera.

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APPENDIX 1: LIST OF SPECIES AND ANALYZED MATERIALS

| Species | Vouchers | Locality |
|--|---|--|
| <i>Blotiella lindeniana</i> (Hook.) R.M.Tryon | Schwartzburd & Becari-Viana 3348 (VIC) Schwartzburd & Becari-Viana 3401 (VIC) Schwartzburd & Becari-Viana 3414 (VIC) | Brazil Brazil Brazil |
| <i>Dennstaedtia</i> (Patania) <i>dissecta</i> (Sw.) T.Moore | Yañez & Marquez 104 (VIC) | Argentina |
| <i>Dennstaedtia</i> (Patania) <i>cornuta</i> (Willd.) T.Moore | Schwartzburd & Becari-Viana 2981 (VIC) Schwartzburd & Becari-Viana 3070 (VIC) Becari-Viana & Pereira 10 (VIC) Becari-Viana & Pereira 11 (VIC) Becari-Viana & Pereira 12 (VIC) | Brazil Brazil Brazil Brazil Brazil |
| <i>Dennstaedtia globulifera</i> (Poir.) Hieron. | Schwartzburd & Becari-Viana 3069 (VIC) Becari-Viana & Pereira 06 (VIC) Becari-Viana & Pereira 07 (VIC) Becari-Viana & Pereira 08 (VIC) | Brazil Brazil Brazil Brazil |
| <i>Dennstaedtia scabra</i> (Wall.) T.Moore | Yamamoto 2636 (VIC,TNS) Yamanaka (VIC-44.535, TNS) | Japan Japan |
| <i>Dennstaedtia</i> (Fuziifilix) <i>hirsuta</i> (Sw.) Mett. ex Miq. | Takesako 4188 (VIC,TNS) Shimozono (VIC-44.536, TNS) | Japan Japan |
| <i>Dennstaedtia</i> (Coptodipteris) <i>wilfordii</i> (T.Moore) Christ. | Yuzawa 3494 (TNS) Saito (VIC- 44.526, TNS) Nagase (VIC-44.538, TNS) | Japan Japan Japan |
| <i>Histiopteris incisa</i> (Thunb.) J.Sm. | Becari-Viana & Pereira 19 (VIC) Becari-Viana & Pereira 20 (VIC) Becari-Viana & Pereira 20 (VIC) Enzat 141 (CRH) | Brazil Brazil Brazil New Zealand |
| <i>Hypolepis acantha</i> Schwartzb. | Schwartzburd et al. 2264 (VIC) | Brazil |
| <i>Hypolepis mitis</i> Kunze ex Kuhn | Schwartzburd & Becari-Viana 3021 (VIC) Schwartzburd & Becari-Viana 3097 (VIC) Becari-Viana & Pereira 03 (VIC) Becari-Viana & Pereira 04 (VIC) Becari-Viana & Pereira 05 (VIC) Schwartzburd & Becari-Viana 3316 (VIC) | Brazil Brazil Brazil Brazil Brazil Brazil |
| <i>Hypolepis punctata</i> (Thunb.) Mett. ex Kuhn | Tsutsui 5135 (VIC, TNS) | Japan |
| <i>Hypolepis rugosula</i> subsp. <i>pradoana</i> Schwartzb. | Becari-Viana & Pereira 01 (VIC) | Brazil |
| <i>Hypolepis stolonifera</i> var. <i>nebularis</i> Schwartzb. | Becari-Viana & Pereira 13 (VIC) Becari-Viana & Pereira 15 (VIC) Becari-Viana & Pereira 14 (VIC) | Brazil Brazil Brazil |
| <i>Hypolepis stolonifera</i> Fée var. <i>stolonifera</i> | Labiak et al. 4269 (VIC) | Brazil |
| <i>Microlepis marginata</i> (Panz.) C.Chr. | Iwatsuki & Kato 79 (CRH) Wang et al. 368 (CRH) Haruda 366 (VIC,TNS) Takesako 5597 (VIC, TNS) | Japan China Japan Japan |
| <i>Microlepis izu-peninsulae</i> Sa.Kurata | Nakaike (CRH- 239661) | Japan |
| <i>Microlepis obtusiloba</i> Hayata | Iwatsuki & Kato 51 (CRH) | Japan |
| <i>Microlepis speluncae</i> (L.) T.Moore | Schwartzburd & Becari-Viana 3290 (VIC) Schwartzburd & Becari-Viana 3310 (VIC) Schwartzburd & Becari-Viana 3315 (VIC) | Brazil Brazil Brazil |

APPENDIX 1: Continued.

| Species | Vouchers | Locality |
|--|---|---|
| <i>Microlepia strigosa</i> (Thunb.) C.Presl | Croft 836 (CRH) Braithwaite 2491 (CRH) Hovell (CRH-465258 A) Kido 12966 (VIC, TNS) | Papua New Guinea New Zealand New Zealand Japan |
| <i>Oenotrichia maxima</i> (E.Fourn.) Copel. | Brownlie 200 (CRH) | New Caledonia |
| <i>Paesia glandulosa</i> (Sw.) Kuhn | Schwartsburd & Fortuna-Perez 2929 (VIC) Schwartsburd & Fortuna-Perez 2930 (VIC) Schwartsburd & Fortuna-Perez 2931 (VIC) | Brazil Brazil Brazil |
| <i>Paesia rugosula</i> (Labill.) Kuhn | More & Brownlie 463 (CRH) | New Caledonia |
| <i>Paesia scaberula</i> (A.Rich.) Kuhn | Smith 84 (CRH) Moore (CRH-533179) | New Zealand New Zealand |
| <i>Paesia tahitensis</i> Copel. | Sykes 446 (CRH) | French Polynesian |
| <i>Pteridium arachnoideum</i> (Kaulf.) Maxon subsp. <i>arachnoideum</i> | Schwartsburd et al. 2838 (VIC, NSW) Schwartsburd et al. 2837 (VIC) Schwartsburd et al. 3383 | Brazil Brazil Brazil |
| <i>Pteridium arachnoideum</i> subsp. <i>campestre</i> (Schrad.) Schwartsb. & P.L.R. Moraes | Alves da Silva et al. 01 (VIC, NSW). | Brazil |

Studies on Oogenesis of the Fern *Lygodium japonicum*

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ABSTRACT.—Oogenesis of *Lygodium japonicum* (Thunb.) Sw. is studied using TEM and cytochemical techniques. The early development of the archegonia of *L. japonicum* is similar to those of the core-leptosporangiate ferns. A mature archegonium always contains an egg, a ventral canal cell (VCC) and a binuclear neck canal cell (NCC). With the development of the egg, a separation cavity forms between the egg and the VCC. However, a pore region, with a diameter of about 3.5 μm , constantly connects the egg and the VCC. The canal cells degenerate gradually accompanied by the accumulation of mucilaginous secretions around the canal cells. PAS reaction reveals that these secretions are polysaccharide in nature. Later development of the archegonia of *L. japonicum* differs greatly from the core-leptosporangiate ferns. The egg nucleus becomes highly irregular and two types of nuclear evaginations are formed during oogenesis. When the egg matures, no egg envelope or fertilization pore are formed. Only a layer of amorphous material is deposited on the outer surface of the egg. This is the first observation of a fern species that has a pore region but does not result in the formation of a fertilization pore. The pore region appears to determine the formation of the fertilization pore. These observations are consistent with the hypothesized phylogenetic position of *L. japonicum* relative to the core leptosporangiate ferns.

KEY WORDS:—fern, *Lygodium japonicum*, pore region, oogenesis.

Analyses of oogenesis in fern species representing the core leptosporangiate ferns including *Ceratopteris richardii* Brongn. (Lopez-Smith and Renzaglia, 2008), *Ceratopteris thalictroides* (L.) Brongn. (Cao et al. 2009, 2010a), *Adiantum flabellulatum* L. (Cao et al. 2010b), *Plagiogyria euphlebia* (Kunze) Mett. (Cao et al. 2011), *Pteridium aquilinum* (L.) Kuhn (Cao et al. 2012b), *Coniogramme emeiensis* Ching & K. H. Shing (Wang et al. 2012a), *Cibotium barometz* (L.) J.Sm. (Wang et al. 2012b), *Anisocampium sheareri* (Baker) Ching (Yang et al. 2013), *Phymatosorus hainanensis* (Nooteboom) S.J. Lu (Zou et al. 2014) demonstrate that all mature eggs of these species possess an egg envelope consisting of a reticular or multilayered structure. Further, all of these species feature a fertilization pore that forms in the center of the upper surface of the egg envelope (Cao et al. 2009, 2010a, 2010b, Cao et al. 2011, Cao et al. 2012b; Wang et al. 2012a, b; Yang et al. 2013, Zou et al. 2014). In contrast, the mature egg of *Osmunda japonica* Thunb., a lineage that is sister to the leptosporangiate ferns (Pryer et al. 2004), possesses neither an egg envelope nor a fertilization pore (Cao et al., 2012a). *Equisetum arvense* L., an even more ancient lineage than *Osmunda* (Pryer et al. 2004, Smith et al. 2006), also has no egg envelope or fertilization pore (Cao et al., 2015). Within the core

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leptosporangiate lineage, the basal members of that clade include such genera as *Lygodium* and *Diplopterygium*. Whether species in these genera produce egg envelopes and fertilization pores is still unclear. Given these facts, we chose to study the cytological features of *L. japonicum*. Our results contribute to understanding the origin and evolution of the egg envelope and fertilization pore in the ferns.

MATERIALS AND METHODS

Spores of *Lygodium japonicum* were collected from plants in Ningbo, Zhejiang province, China. The spores were surface sterilized with a 5% sodium hypochlorite solution for a total of 3 min. After rinsing three times with distilled water, the spores were sown on a modified Knop's solution (0.8g $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$; 0.2g KH_2PO_4 ; 0.2g KNO_3 ; 0.2g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, dissolved in 1 liter of distilled water), solidified with 1.5% agar in culture dishes. These dishes were placed in an artificial climate chamber at 25°C in the light (18 h) and 20°C in the dark (6 h). After 4 to 5 weeks, archegonia developed on the lower surface of the gametophytes just behind the meristematic region.

Gametophytes bearing various stages of archegonia were immersed in 3% glutaraldehyde in 0.1 mol/L phosphate buffer at room temperature for 6-12 h. The specimens were subsequently washed three times with the same buffer, fixed in 2% aqueous osmium tetroxide for 2 h, rinsed three times in the same buffer and embedded in Spurr's resin (SPI-Chem, USA) via a graded acetone series. Specimens were thick sectioned for the presence of the archegonia and thin-sectioned with a diamond knife on an ultramicrotome (Leica EM UC7, Germany). For periodic acid Schiff (PAS) reactions, sections (1 μm thick) were placed in drops of water on glass slides and allowed to adhere to the glass by drying at 50°C. The thick sections were first immersed in water and then in a 1% aqueous solution of periodic acid for 30 min. The sections were washed to remove any traces of periodic acid prior to the application of Schiff's reagent for 30 min. The Schiff's reagent was rinsed off with sulfurous acid solution. The stained sections were washed under running water for about 5 min. All the sections were examined using a light microscope (Nikon E-800) and photographed with DXM1200 digital camera. For the ultrastructural observation, the thin sections were stained with uranyl acetate and lead citrate. All specimens were observed with an electron microscope (Tecnai G2 Spirit BioTWIN, FEI Company).

RESULTS

The Recently Initiated Egg

When an egg is initiated, the archegonium contains an egg cell, a ventral canal cell (VCC), and a bi-nucleate neck canal cell (NCC) (FIG. 1a). These three inner cells are closely appressed to the wall of the jacket cells of the archegonium (FIG. 1a). There are well-developed plasmodesmata between the NCC and the

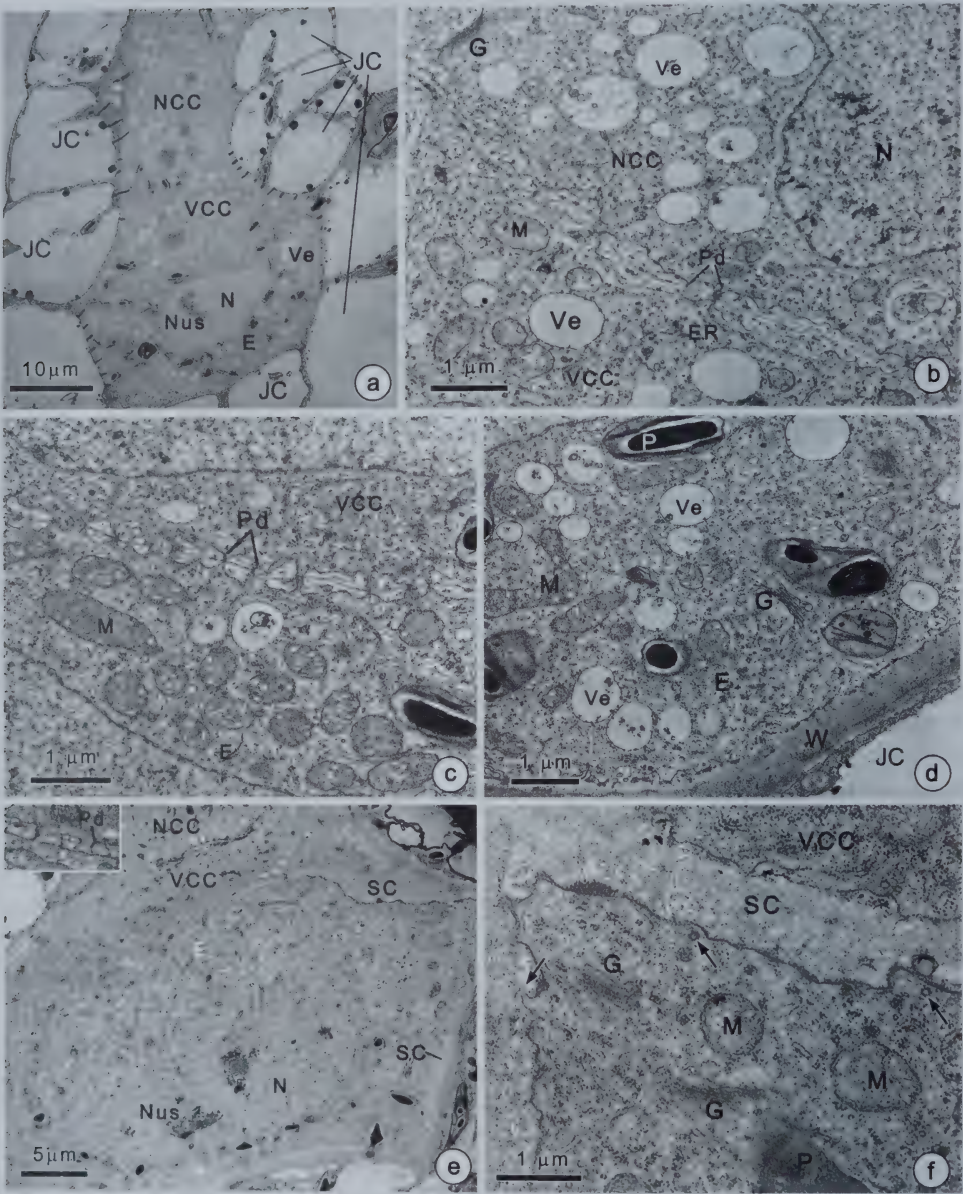


FIG. 1. RECENTLY INITIATED EGG. (a) An archegonium containing a recently initiated egg (E), a ventral canal cell (VCC) and a neck canal cell (NCC). (b) Plasmodesmata (Pd) connect the VCC and the NCC. (c) Plasmodesmata (Pd) connect the egg and the VCC. Abundant mitochondria (M) in the egg cytoplasm. (d) Side of the egg showing organelles, including plastids (P), Golgi bodies (G) and mitochondria (M). (e) A separation cavity (SC) forms at the periphery and lower side of the egg. Plasmodesmata (Pd) connect the egg and the VCC in the central part (inset). (f) Abundant Golgi bodies (G) occur in the upper side of the egg. Small vesicles (arrows) are seen in the periphery of the egg. JC, jacket cell; N, Nucleus; Nus, nucleolus; Ve, vesicle; W, wall.

VCC, also between the VCC and the egg (FIG. 1b, c), but plasmodesmata are absent between these inner cells and the jacket cells of the archegonium (FIG. 1d). The nucleus of the recently initiated egg is nearly oblong and contains irregular nucleoli (FIG. 1a). Vesicles, with a diameter of about 0.5-1 μ m, are distributed principally along the lateral side of the egg cytoplasm (FIG. 1a, d). Plastids in the egg cytoplasm contain one or two starch grains and photosynthetic lamellae (FIG. 1d). Mitochondria are distributed throughout the cytoplasm of the egg, but some of them are clustered in the upper part of the egg cytoplasm (FIG. 1c). Golgi bodies can be seen at the periphery of the egg cytoplasm (FIG. 1d). Organelles in the canal cells are similar to those in the egg (FIG. 1b, c).

Maturing Egg Stage

FORMATION OF THE SEPARATION CAVITY.—The first detectable change of the egg during maturation is the formation of a separation cavity. The separation cavity is initially formed at the periphery of the upper surface of the egg and the lower side of the egg (FIG. 1e). The plasmalemma of the egg becomes dissociated from the wall in the periphery and lower side of the egg (FIG. 1e). However, plasmodesmata still connect the egg and the VCC in the central region (FIG. 1e, inset). Golgi bodies become more abundant at the upper side of the egg. Some small vesicles (probably Golgi vesicles) are frequently viewed beside the plasmalemma (FIG. 1f, arrows). The nucleus of the egg becomes flattened with a depressed upper surface (FIG. 1e).

Subsequently, the separation cavity above the egg expands centripetally while the connection region decreases correspondingly to a diameter of about 3.5 μ m (FIG. 2a-c). Such a connection region is called the pore region. There are plasmodesmata in the pore region connecting the egg and the VCC (FIG. 2c). The amorphous materials, which show a weak positive PAS reaction, are seen in the separation cavity around the egg and the canal cells (FIG. 2a). The nucleus of the egg becomes somewhat bowl-shaped (FIG. 2b). Numbers of vesicles decrease along the lateral side while increasing along the upper part of the egg cytoplasm (FIG. 2b). Plastids still contain starch grains, but the photosynthetic lamellae have disappeared (FIG. 2c, d). At this stage, the vesicles and Golgi bodies increase greatly in the cytoplasm of the canal cells (FIG. 2c, f). Plasmodesmata connect the VCC and NCC consistently (FIG. 2e).

DEGENERATION OF THE CANAL CELLS AND FORMATION OF THE NUCLEAR EVAGINATIONS.—The most prominent feature of the archegonium at this stage is the degeneration of the canal cells. The mucilaginous secretions, showing a strong positive PAS reaction, are formed around the VCC and NCC (FIG. 3a), but the secretions in the separation cavity around the egg show a weak positive PAS reaction (FIG. 3a, asterisks). The mucilaginous secretions beside the VCC appear to have differently stained features: the inner layer of the mucilaginous secretions is lightly stained (FIG. 3a, arrowhead), but the outer layer is deeply stained (FIG. 3a, arrow). Under electron microscope, the inner layer of the

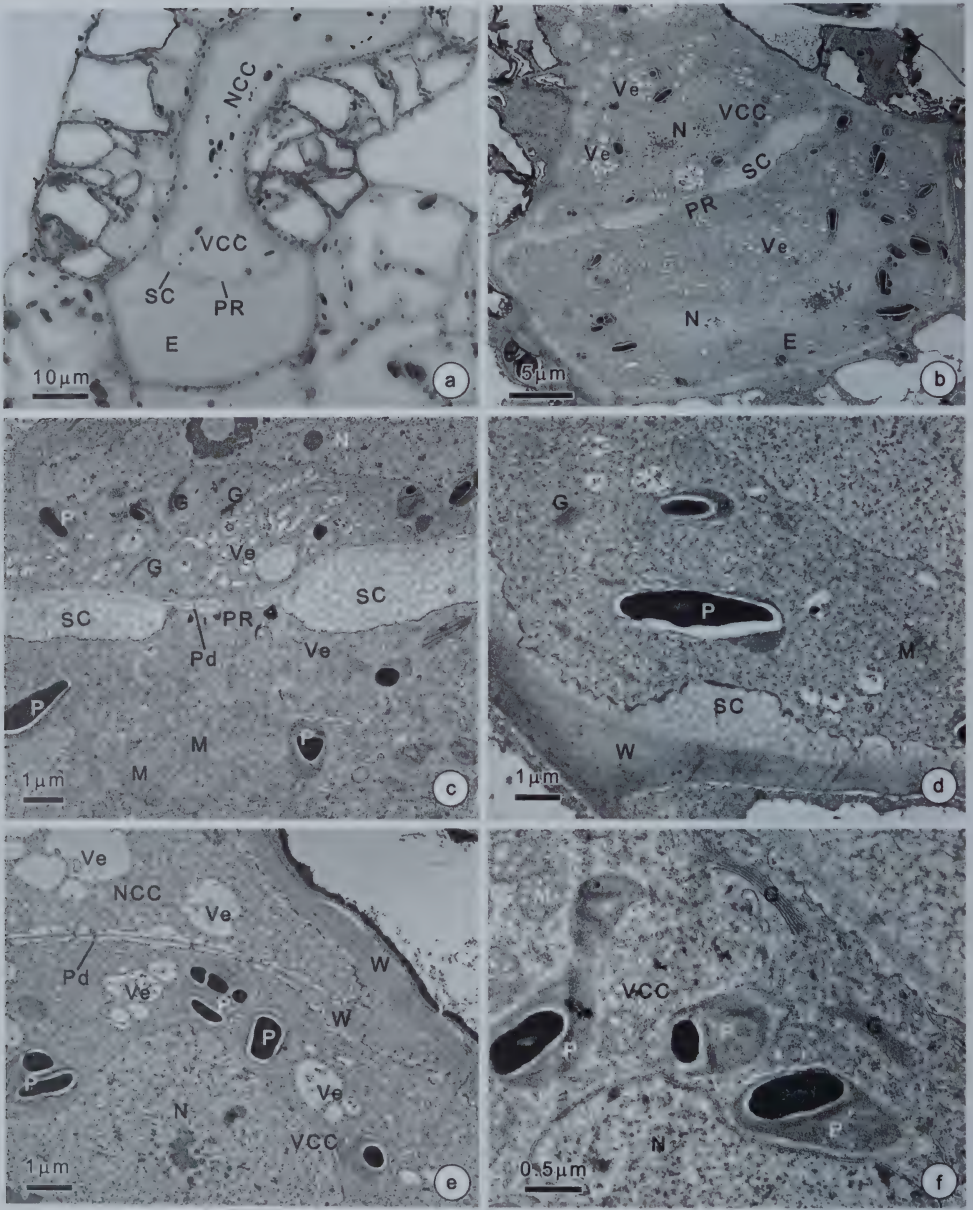


FIG. 2. MATURING EGG STAGE I. (a) PAS reaction of a maturing archegonium, a separation cavity (SC) is formed around the egg (E). A pore region (PR) connects the egg and VCC. (b) The archegonium of the same stage as in Figure 2a under TEM, showing the pore region (PR) and separation cavity (SC). (c) Magnification of Figure 2b showing the pore region (PR) and Plasmodesmata (Pd) in the pore region. (d) Lower side of the egg showing organelles, including plastids (P), Golgi bodies (G) and mitochondria (M). (e) Part of the VCC and NCC, showing the Plasmodesmata (Pd) and vesicles (Ve). (f). Organelles in the upper part of NCC, Golgi bodies (G) are active. N, Nucleus; W, wall.

mucilaginous secretions contains fine granules (FIG. 3b, arrowhead), while the outer layer contains coarse granules (FIG. 3b, arrow).

The egg cell remains connected with the VCC at the pore region in this stage (FIG. 3a-c). In contrast to the previous stage, the nucleus becomes cup-shaped with an irregular surface (FIG. 3b). Nuclear evaginations, which look like bubbles, are formed on the surface of the nucleus (FIG. 3d). Most evaginations remain connected to the main body of the nucleus (FIG. 3d). However, evaginations, without connection to the nucleus body, can also be seen near the nuclear body (FIG. 3b). The matrix of the evaginations resembles that of the main body of the nucleus (FIG. 3d). A thin layer of amorphous materials is deposited on the outer surface of the plasmalemma (FIG. 3e, arrows). At this stage, the originally spherical or ellipsoidal plastids start to stretch, curve, and enclose a part of the cytoplasm (FIG. 3d, f). Occasionally, a mitochondrion is enclosed by the deformed plastid (FIG. 3d). Plastoglobuli can be seen in the plastids (FIG. 3f). However, the plastids in the VCC, different from those in the egg, remain unchanged (FIG. 3f). Golgi bodies and endoplasmic reticula increase prominently in the upper part of the egg (FIG. 3c, e), while amorphous materials become obvious in the separation cavity (FIG. 3e). Vesicles are mainly located in the upper part of the egg (FIG. 3b).

Mature Egg Stage

At this stage, almost all cytoplasm of the canal cells has decomposed into mucilaginous secretions. These secretions show a strong positive PAS staining (FIG. 4a). However, the amorphous materials in the separation cavity around the mature egg show a relatively weak positive PAS staining (FIG. 4a, arrows). Under the electron microscope, these amorphous materials are evident (FIG. 4b, c, arrows). Sometimes a block of electron-opaque material lies between the ventral canal cell and the egg cell (FIG. 4b, c, asterisks). At the mature stage, the VCC has been detached from the egg. No pore region is found in the mature egg. Magnification shows that a layer of amorphous materials is deposited on the outer surface of the egg plasmalemma (FIG. 4d, arrows). All the specimens examined via the electron microscope show that no typical egg envelope (formed by the core-leptosporangiate ferns) is formed in the mature egg of *Lygodium japonicum*.

The nucleus of the egg remains highly irregular in shape (FIG. 4e). In contrast to the former stage, the evaginations are different: in this stage, evaginations are surrounded by two layers of nuclear envelope. There are opaque materials between the two nuclear envelopes (FIG. 4e). Serial sections show that the evaginations are formed by small nuclear bodies that are already formed inside the nucleus (FIG. 4f, asterisks). These small nuclear bodies move outwards, are wrapped by the nuclear envelope and ultimately form the complicated nuclear evaginations. Some evaginations remain connected to the main body of the nucleus via narrow isthmuses (FIG. 4e). However, the same kind of evaginations with no connection to the nucleus body can also be observed in the cytoplasm of the egg (FIG. 4e). The plastids degenerate further and no starch grains or photosynthetic lamella are observed (FIG. 4e). However, the

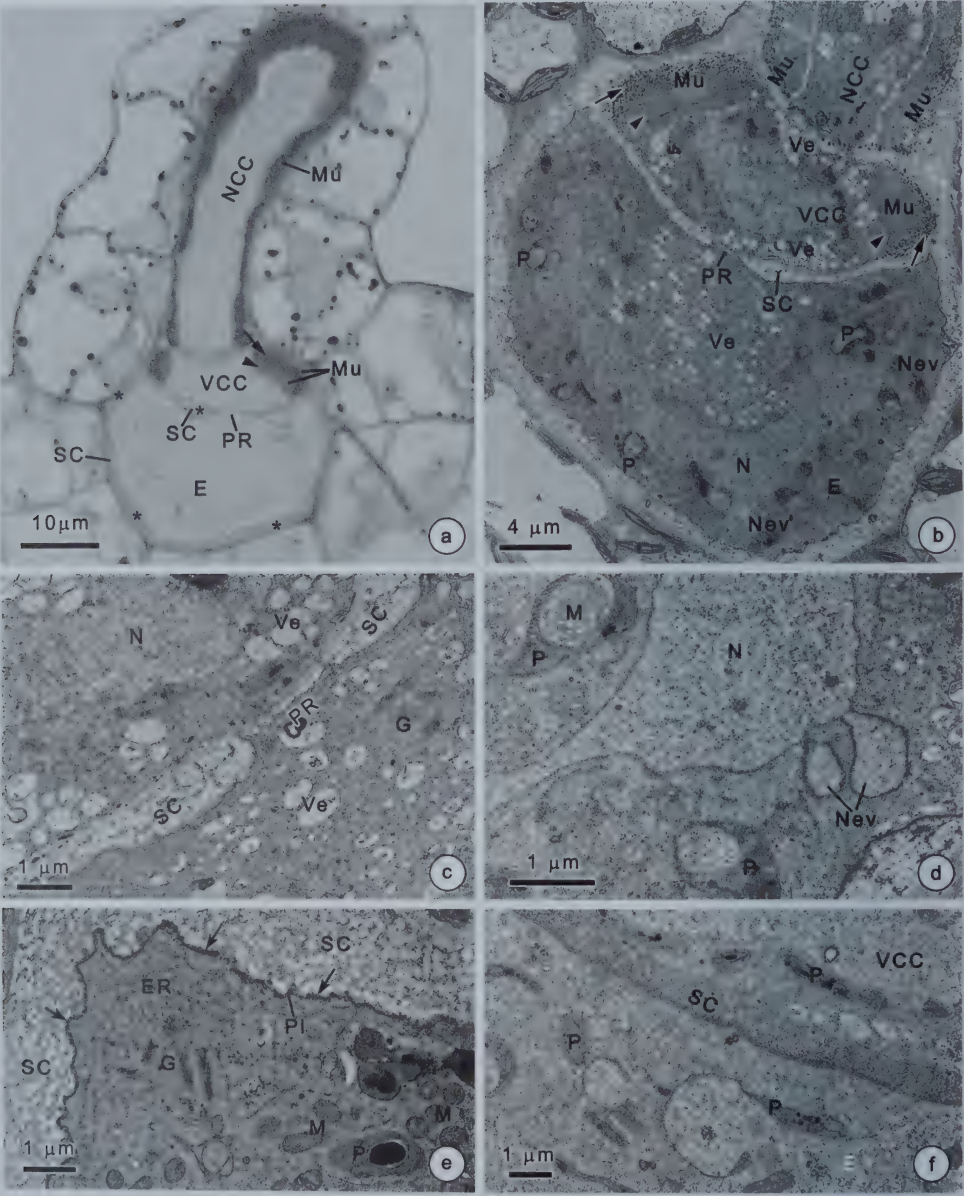


FIG. 3. MATURING EGG STAGE II. (a) PAS reaction of a maturing archegonium at a late stage, mucilaginous secretions (Mu) show strong positive PAS reaction around the VCC and NCC. The secretions in the separation cavity (SC) show a weak PAS reaction (asterisks). The inner layer of the mucilaginous secretions shows weak staining (arrowhead), but the outer layer is deeply stained (arrow). (b) The same archegonium as Figure 3a under TEM. Vesicles converge in the upper part of the egg (E). Plastids (P) around the nucleus (N) enclose a part of the cytoplasm. (c) Magnification of Figure 3b showing the pore region (PR). (d) Part of the egg showing nuclear evaginations (Nev). Plastids (P) enclose a part of the cytoplasm. (e) Upper side of the egg showing abundant Golgi bodies (G), endoplasmic reticulum (ER). Amorphous material is deposited on the outer surface of the egg (arrows). (f) Part of the egg and the VCC, elongated plastids (P) is enclosing the egg cytoplasm. M, mitochondria; PL, plasmalemma.

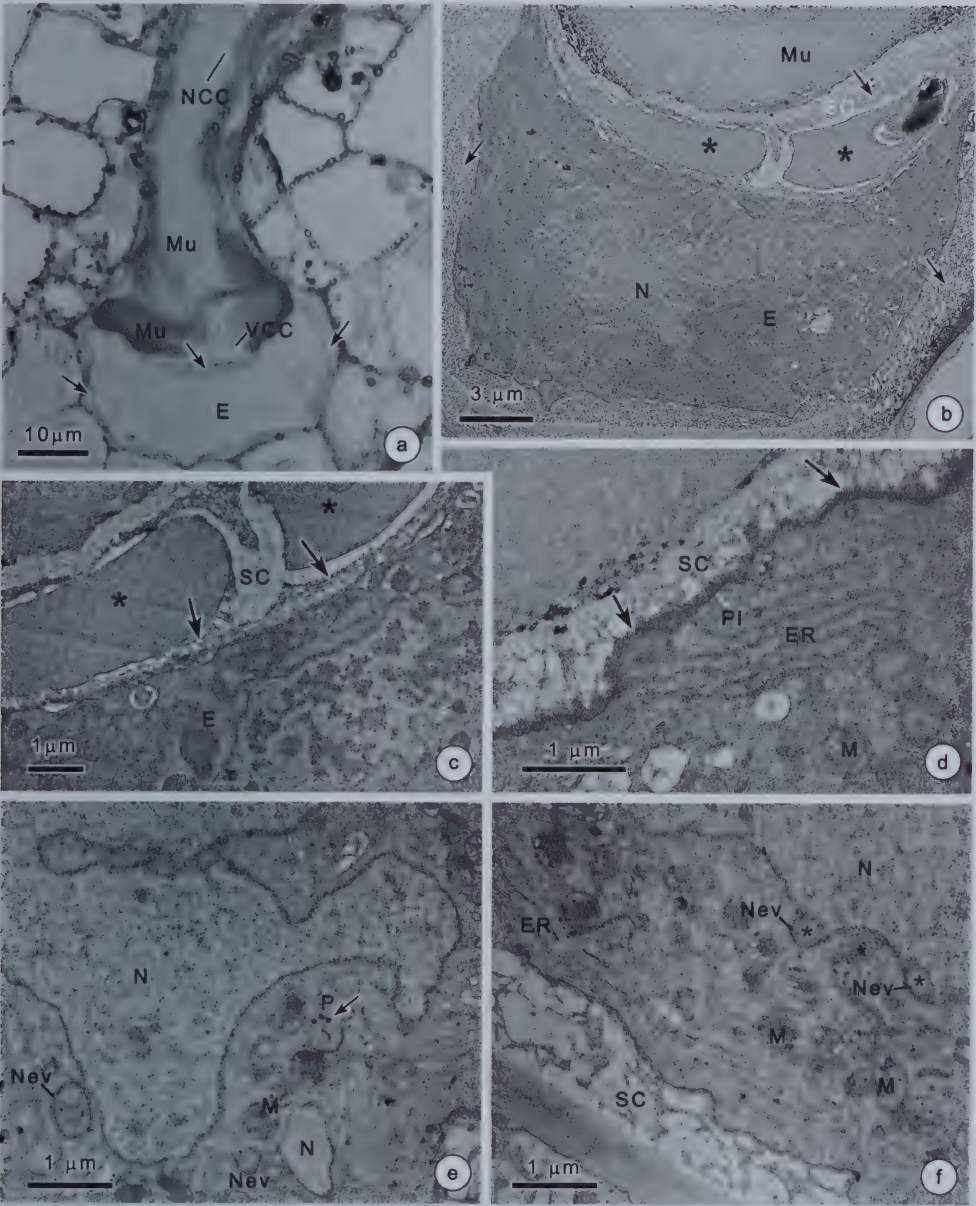


FIG. 4. MATURE EGG. (a) PAS reaction showing almost all the cytoplasm of the VCC and NCC has been converted into mucilaginous secretions (Mu). (b) The archegonium at the same stage as Figure 4a under TEM. Amorphous material (arrows) in the separation cavity (SC) and an electron-opaque material (asterisks) is seen between the VCC and the egg. (c) Magnification of Figure 4b showing the upper part of the egg. (d) Another mature egg, showing the deposition of amorphous material (arrows). (e) Part of the mature egg, showing the irregular nucleus and the complicated nuclear evaginations (Nev). The plastids (P) degenerate, only containing plastoglobuli (arrow). (f) The mature egg showing that the evaginations are formed by small nuclear bodies (asterisks). E, Egg; ER, endoplasmic reticula; M, mitochondria; N, Nucleus.

plastids can be identified by the presence of plastoglobuli (FIG. 4e, arrow). The well-developed mitochondria possess clear cristae (FIG. 4d-f). Endoplasmic reticula are frequently seen in the periphery of the mature egg (FIG. 4f).

DISCUSSION

The fertilization pore, a structure which limits the penetration of the sperm, was first discovered in the fern *Ceratopteris thalictroides* (Cao et al. 2009). All the core-leptosporangiate ferns, so far examined, possess a fertilization pore and an egg envelope in the mature egg (Cao et al. 2009, 2010a, 2010b, Cao et al. 2011, Cao et al. 2012a,b; Wang et al. 2012b; Yang et al. 2013, Zou et al. 2014). However, *Equisetum arvense* and *Osmunda japonica*, which are sister lineages to the leptosporangiate ferns, do not form a fertilization pore during oogenesis (Cao et al. 2012a, Cao et al. 2015). We have been surveying other species to elucidate the origin of the fertilization pore. The genus *Lygodium* is phylogenetically basal among the core leptosporangiate ferns (Smith et al., 2004). Ultrastructural and cytological observations demonstrate that *L. japonicum* forms a pore region between the egg and the ventral canal cell during oogenesis, whereas no egg envelope or fertilization pore are formed in the mature egg. In this aspect, *Lygodium* differs from the core-leptosporangiate ferns whose egg envelope and fertilization pores are formed through the following three stages: (1) A separation cavity forms between the egg and VCC. (2) The pore region forms and persistently connects the VCC and the egg. (3) The egg envelope forms and the lack of an egg envelope in the pore region finally leads to the production of the fertilization pore (Cao et al. 2010a, b, 2011, 2012b; Yang et al. 2013; Zou et al. 2014). By examining the three stages of the fertilization pore formation in the core leptosporangiate ferns, the pore region is considered to be a key structure which determines the site and the size of the fertilization pore (FIG. 5, a1-a3). However, only the first two stages of the fertilization pore formation of the core-leptosporangiate ferns occur in *Lygodium*. The fact that *Lygodium* only forms a pore region with no formation of an egg envelope or fertilization pore indicates that the emergence of the pore region is a precondition for the origin of the fertilization pore (FIG. 5, b1-b3). Therefore, the present investigation provides evidence for understanding the evolution of the fertilization pore.

So far, it has been found that all the core-leptosporangiate ferns form an elaborate egg envelope in the mature egg. However, the egg envelopes of the core-leptosporangiate ferns are different in structure. Most ferns (except Pteridaceae), including the *Pteridium aquilinum*, *Plagiogyria euphlebia*, *Anisocampium sheareri* and *Phymatosorus hainanensis*, form a reticular egg envelope by the deposition of amorphous material in the separation cavity on the outer surface of the egg plasmalemma (Cao et al. 2011, 2012b; Yang et al. 2013, Zou et al. 2014). Hence, there is no amorphous material in the separation cavity when the egg matures. The amorphous material appears to be involved in forming the egg envelope (Cao et al. 2011, 2012b). In the members of the family Pteridaceae, including *Ceratopteris thalictroides*,

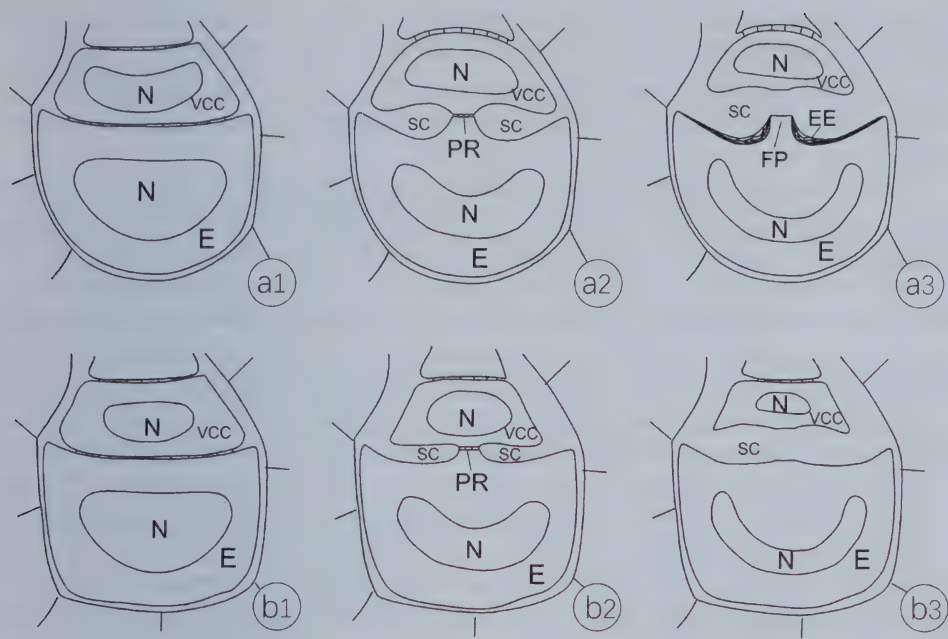


FIG. 5. DIAGRAMS OF OOGENESIS OF THE CORE- LEPTOSPORANGIATE FERNS (a1-a3) AND *LYGODIUM* (b1-b3). (a1) Young egg (E) stage. (a2) Separation cavity (SC) and pore region (PR) of the maturing egg stage. (a3) Mature egg with egg envelope (EE) and fertilization pore (FP). (b1) Young egg (E) stage. (b2) Separation cavity (SC) and pore region (PR) of the maturing egg stage. (b3) Mature egg lacking an egg envelope. N, Nucleus; VCC, ventral canal cell.

Adiantum flabellulatum and *Coniogramme emeiensis*, the egg envelopes appear to be formed by the attachment of the ER on the inner surface of the egg plasmalemma, resulting in the formation of a multilayered egg envelope. Given that no amorphous material is consumed to form the egg envelope, abundant amorphous material remains in the separation cavity when the egg is mature (Cao *et al.* 2008, 2010a, b; Wang *et al.* 2012b). In the present investigation, even though no egg envelope is formed in the mature egg of *L. japonicum*, there is still a thin layer of amorphous material deposited on the outer surface of the mature egg. However, the layer is too thin to consume all the amorphous material in the separation cavity when the egg matures. Moreover, a block of electron-opaque material also remains in the separation cavity, which is similar to that in members of the Pteridaceae, including *Adiantum flabellulatum* and *Coniogramme emeiensis* (Cao *et al.* 2010b; Wang *et al.* 2012a). This evidence supports a close relationship between *Lygodium* and members of the Pteridaceae. Our cytological observations about oogenesis support some traditional viewpoints that the Schizaeaceae *sensu lato* (including *Lygodium*) is ancestral to the family Pteridaceae (Smith *et al.*, 1995).

In the core-leptosporangiate ferns, a naked region (the pore region) remains in the egg envelope when the ventral canal cell separates from the egg. In the

present investigation, no such naked region is observed in the mature egg via electron microscope. However, there is still a region that stains weakly in toluidine blue in the upper surface of the mature egg under light microscope observation (unpublished data). The ultrastructure of the weakly stained region was not observed in this investigation.

The nuclear behavior of *L. japonicum* is complicated. The nucleus becomes highly irregular and two types of nuclear evaginations occur as the egg matures. The simple evaginations are bubble-like and appear to be produced by protrusions of the nuclear envelope. This type of evagination can be observed in other ferns, for example, *Adiantum flabellulatum*, *Plagiogyria euphlebia*, *Pteridium aquilinum*, *Coniogramme emeiensis*, *Cibotium barometz*, *Phymatosorus hainanensis* (Cao et al. 2010b; 2011; 2012b; Wang et al. 2012a, b; Zou et al. 2014). *Lygodium japonicum* also produces another type of nuclear evagination that is formed by small bodies inside the nucleus. This complicated nuclear evaginations resemble those of *Anisocampium shearerii* (Yang et al. 2013). The function and significance of the nuclear evaginations in *L. japonicum* are still obscure.

The cytoplasmic organelles of *L. japonicum* are similar to those of the core leptosporangiate ferns (Cao et al. 2009; 2010a, b; 2011; 2012b; Wang et al. 2012; Yang et al. 2013; Zou et al. 2013). However, the plastids in the egg of *L. japonicum* show complicated behaviors at the late stage of the maturing egg. These plastids always enclose a part of the cytoplasm. The special cytological behaviors of the plastids have not been seen in other ferns before and the biological significance of these behaviors remains unclear. However, these plastids finally return to their original oval shape in the mature egg.

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Spore Production and Dispersal in Two Temperate Fern Species, With an Overview of the Evolution of Spore Production in Ferns

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ABSTRACT.—The presence of a nutritionally independent gametophyte generation of spore-producing land plants, including those of ferns, has often been cited as an important factor in explaining their broader geographic distribution at the level of species and genera relative to that of seed plants. Dispersing spores widely and successfully is critical to establishing populations—both in terms of production and dispersal distance—yet literature with direct observation of these variables is scant. We double the number of studies directly observing dispersal in fern species by conducting spore trap experiments on *Adiantum pedatum* and *Deparia acrostichoides*, which grow together at a site in southeastern Ohio, U. S. A. In interpreting these results, we summarize the literature on spore production in ferns and examine the contribution of phylogenetic history to the variation in spore production across ferns. We corroborate findings that the vast majority of spores produced are dispersed within 2 m of the parent plant. Additionally, spore production in ferns varies widely between species but shows some phylogenetic conservatism and is correlated to frond area. We conclude that gametophyte (and sporophyte) establishment over distances greater than 3 m is governed by rare spore dispersal, but the sheer number of spores produced increases the probability of this event occurring and the establishment of sporophytes is likely dependent upon gametophytic traits. In ferns as a whole, differences in spore production are related to differences in frond area, but may also be related to overcoming genetic complications involved in long-distance dispersal.

KEY WORDS.—reproductive output; gametophyte; long-distance dispersal

The ability of an organism to disperse not only buffers against species extinction due to chance events, but also allows exchange of genetic material between distantly related genotypes and potentially reduces intraspecific competition. A strong selective value for the dispersal of propagules is clearly illustrated in the various elaborations of seeds and fruits in angiosperms (Howe and Smallwood, 1982). In the geologic scale, the ability of genetic material to be dispersed widely within relatively short periods of time is demonstrated in Holocene pollen records of angiosperms and gymnosperms, with estimates of dispersal rates of up to 1000 m/yr (Bernabo and Webb, 1977; Clark, 1998), although molecular data suggest slightly slower migration rates of >100 m/yr (McLachlan, Clark, and Manos, 2005). Observations of fungal spores and plant pollen at altitudes exceeding 1350 m (Newman, 1948)

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confirms the ability of microscopic propagules to disperse widely. In ferns, spores appear viable even after exposure to increased ultraviolet light at these altitudes (Wolf, Schneider, and Ranker, 2001). The importance of long-distance dispersal is well-illustrated in the colonization of oceanic islands, with classic studies on islands such as Krakatau demonstrating that establishment by propagules dispersed long distances can happen in less than 50 years (Cain, Milligan, and Strand, 2000).

Spore producing lineages such as ferns, therefore, should be able to disperse widely (Tryon, 1970). In addition, the presence of a free-living gametophyte that grows from a spore, instead of the nutritionally dependent gametophyte in pollen, should facilitate widespread establishment of sporophytes. The widespread distributions of many genera and species of bryophytes and ferns, as compared to seed plant distributions, has frequently been attributed to this hypothesized ability to both readily disperse spores and establish gametophytes (Barrington, 1993; Kramer, 1993; Schofield and Crum, 1972; Shaw, 2001; Smith, 1972). Differences in the distribution of fern and seed plant genera may include the: (1) older age of ferns, (2) slower rates of evolution in ferns, (3) non-equivalency of taxonomic ranks, and (4) higher dispersal ability of ferns (Smith, 1972). Molecular evidence has soundly rejected the first reason (Schneider et al., 2004), the third reason is somewhat intractable, and the second reason has not been fully addressed. Investigations into the importance of long-distance dispersal in ferns has greatly benefited from molecular systematics and fossil calibration of molecular clocks. These studies suggest that fern distributions have been driven by both vicariance and long-distance dispersal (Geiger et al., 2007; Korall and Pryer, 2014; Ranker, Floyd, and Trapp, 1994; Sessa, Zimmer, and Givnish, 2012). Currently, long-distance dispersal continues to be implicated as a major reason for the widespread distribution of fern genera and species.

Whereas indirect observations of the importance of long-distance dispersal in fern biogeography exist, few empirical studies exist that examine how frequently spores might be dispersed over long distances as measures of both spore output and dispersal capability. In part this is due to the difficulty of measuring structures that are produced in the millions or billions of units by a single individual (Page, 1979), and also in tracking dispersed spores. However, crude measures of both these variables have been made. The most important and thorough study the complete life history of fern species has been that of Peck, Peck, and Farrar (1990). This study, conducted at a site in Iowa, U.S.A., examined the life history of 14 homosporous fern species spanning the breadth of temperate fern diversity. This study examined multiple aspects of life history including measures of spore production and dispersal. They found that between study species, there were vast differences in fecundity (as measured by number of spores produced annually), varying from 54×10^3 spores per plant in *Crypogramma stelleri* (S.G. Gmel.) Prantl to 325×10^6 spores per plant in *Dryopteris goldiana* (Hook.) A. Gray, as well as in percentage of spores actually released per sporangium (7% in *Polypodium virginianum* L. to 100% in *Botrychium virginianum* (L.) Sw.).

In an intensive study of the dispersal pattern of *Botrychium virginianum*, Peck, Peck, and Farrar (1990) found that the vast majority of spores do not disperse beyond 2 m from the parent plant, even when there is little herbaceous canopy that would reduce the strength of the passing air current. These results are consistent with other studies on spore dispersal in ferns. Conant (1978) directly measured spore dispersal of the tree fern *Cyathea arborea* (L.) Sm. by radioactively tagging spores. Although the study plant was 8 m tall, most spores were trapped within a 7.5 m radius of the stem although some spores were detected at 30 m. Roe-Andersen and Southworth (2013) examined dispersal in *Botrychium pumicola* Coville at distances up to 10 m from the parent population. They found spores trapped at up to 10 m but most spores were trapped within 1 m. Such patterns are consistent with similar studies on spore dispersal in bryophytes which have found most spores are trapped at distances of less than 2 m from parent plants (McQueen, 1985; Söderström and Jonsson, 1989; Stoneburner, Lane, and Anderson, 1992; Sundberg, 2005).

A recent study by Flinn (2007) quantified spore production in several temperate species in terms of spore density in the atmosphere, but these measures are based on regression equations rather than direct counts. Furthermore, no estimates of production or dispersal distance for individual plants are provided. Studies on spore banks have reported spores from much greater distances from any plants, up to 50 m from the nearest fern fronds (Penrod and McCormick, 1996; Schneller, 1998). These estimates have been cited as spore dispersal distances (Sheffield, 2008), but it is impossible to ascertain when such spores were deposited or if soil disturbance has led to movement.

In addition to dispersal pattern, Peck, Peck, and Farrar (1990) studied the selfing (spore isolate) potential (equivalent to gametophytic selfing of Haufler et al. (2016)) of fern gametophytes at the same site and found wide intraspecific variation in the ability for gametophytes to self-fertilize. The ability to self-fertilize appeared to be related to local abundance, as sporophytes of *Asplenium platyneuron* (L.) BSP occurred in highly localized patches in the area and its gametophytes were capable of self-fertilization, whereas *Adiantum pedatum* L. had self-incompatible gametophytes (incapable of gametophytic selfing sensu Haufler et al. (2016)) and its sporophytes were ecologically dominant. Based on these observations, Peck, Peck, and Farrar (1990) concluded: "long distance dispersal and colonization [of ferns] are primarily dependent on reproduction via single spores," (p. 136) and that fern dispersal is therefore, "overwhelmingly local" (p. 139). Additionally, they point out that some species of ferns are adapted to rare long distance dispersal, while others are adapted for local dominance. Establishment after long distance dispersal in self-incompatible species may be a possibility for some species via perennial gametophytes and gametophytic gemmae production (Dassler and Farrar, 2001). Recent molecular evidence at the population level has demonstrated inbreeding within fern populations (Chung et al., 2012; De Groot et al. 2012; Jimenez et al., 2010; Keiper and McConchie, 2000). Additionally, laboratory

culture of gametophytes has demonstrated widespread ability for gametophytes to self or interbreed with sibling gametophytes (Sessa, Testo, and Watkins, 2016). These results further suggest that fern dispersal and gametophyte establishment can be highly local and, in the immediate vicinity of the sporophytes, at least at the level of years or decades.

Since the study of Peck, Peck, and Farrar (1990), no similarly executed study has been conducted. Importantly, their study of dispersal distance was only conducted on one species, *Botrychium virginianum*. As the reproductive behavior of Ophioglossaceae with a specialized sporophore may have produced atypical results, sampling of a broader, more representative subset of taxa is needed.

This study adds to the body of knowledge on fern dispersal by measuring spore output and dispersal distance for two fern species representing two major lineages of ferns within the order Polypodiales (PPG1 2016): *Adiantum pedatum* L. (Pteridaceae) and *Deparia acrostichoides* (Sw.) M. Kato (Athyraceae). We hypothesize that, as with spore production, dispersal distance should vary between species. Dispersal distance should be related to frond height, as more elevated sporangia should be more exposed to passing air currents as well as have a longer hang time in the atmosphere. Therefore, we expect that as a result of a greater fertile frond height, *D. acrostichoides* should disperse spores farther than *A. pedatum*, and both should disperse spores farther than *B. virginianum*. However, in both cases, the majority of spores produced should be dispersed close to the parent plant.

As interpretation of studies of ecological and morphological traits dealing with multiple species requires a phylogenetic context that controls for non-independence of species (Felsenstein 1985; Freckleton, Harvey, and Pagel, 2002), we summarize the current and highly preliminary knowledge of the evolution of spore production in ferns, and argue that knowledge of spore production is a critical aspect of understanding the evolutionary ecology of ferns. Given the wide range of variability in spore production (Page, 1979; Peck, Peck, and Farrar, 1990), we expect spore production to be under natural selection and, given the phylogenetic context, we propose several hypotheses that should be further examined in the future.

MATERIALS AND METHODS

Study site and species.—Experiments were conducted at “Deep Woods Farm” (Deep Woods), a roughly 280 acre tract of private land located approximately 1.5 miles southeast of South Bloomingville in Hocking County, Ohio, USA (39.407371° N, 82.576035° W). The site occurs in the unglaciated Allegheny Plateau region of the state and consists of several community types, including deciduous woods, swamp woods, old fields, and hemlock (*Tsuga canadensis* (L.) Carrière) ravines (Riccardi and McCarthy, 2003). Fifteen species of ferns have been recorded at Deep Woods to date (J. Rose, personal observation). Four species, *Adiantum pedatum*, *Deparia acrostichoides*, *Dryopteris intermedia* (Muhl.) A. Gray, and *Polystichum*

acrostichoides (Michx.) Schott are particularly abundant at Deep Woods. We selected two of these species based on their phylogenetic, ecological, and morphological disparity. *Adiantum pedatum* occurs on mesic slopes in both the deciduous forest and hemlock ravines. Because of the steep topography characteristic of hemlock ravines, we restricted our study of *A. pedatum* to individuals in the deciduous mesic forests. Typically associated canopy species include *Acer saccharum* Marsh., *Fagus grandifolia* Ehrh., *Liriodendron tulipifera* L., *Quercus rubra* L., and *Q. alba* L. The understory is dominated by *Hamamelis virginiana* L. and *Lindera benzoin* L. The herbaceous layer is mostly spring geophytes and is virtually nonexistent during sporulation. *Adiantum pedatum* produces sori beneath inrolled frond margins located on horseshoe-shaped fronds and it reproduces clonally by means of spreading rhizomes and generates solitary fronds along its length. This species was selected because it is the most phylogenetically and morphologically distinct common species at Deep Woods and also because it was studied by Peck, Peck, and Farrar (1990), allowing comparison to their estimate of spore production.

The second study species, *Deparia acrostichoides* (Sw.) M. Kato. (Athyriaceae) generally occurs in riparian forests, though it also extends up mesic slopes. Typically associated canopy species include *Betula nigra* L., *Ulmus americana* L., *Carya* spp., *Platanus occidentalis* L., and *Liriodendron tulipifera*. The understory is dominated by *Lindera benzoin*, *Corylus americana* Marsh., and *Alnus rugosa* (L.) Moench. The herbaceous layer is dominated by *Verbesina alternifolia* (L.) Britt., *Elymus* spp., *Onoclea sensibilis* L., and miscellaneous graminoids. The understory layer and herbaceous layer are much denser in the swamp forest compared to the deciduous mesic forest in which *Adiantum pedatum* occurs. *Deparia acrostichoides* produces sori beneath j-shaped, hyaline indusia. Spore-producing fronds are separable from sterile fronds in both their larger size, more erect habit, and less densely spaced pinnae. Of the three most abundant ferns at Deep Woods, *D. acrostichoides* exhibits the greatest ecological differences from *A. pedatum* as both *Polystichum acrostichoides* and *Dryopteris intermedia* co-occur with *A. pedatum* on mesic slopes at Deep Woods.

Spore production estimates.—Collections of fresh, undehisced sporangia were made in the field, preserved in sealed glassine envelopes, and transported back to the lab where they were kept in a refrigerator until counted. Counts were made within a few days of collection. Estimates of spores produced per plant of both *A. pedatum* and *D. acrostichoides* were made by counting the number of spores on portions of the frond and extrapolating to the entire plant. To calculate the number of spores per individual plant in *A. pedatum*, we counted and multiplied together the means of the number of fertile pinnae per frond, total length (mm) of marginal indusium, number of sporangia per millimeter of marginal indusium, and number of spores per sporangium. Because the spreading, non-crown-forming nature of asexual reproduction in *A. pedatum* made delimitation of a single individual exceedingly difficult, we viewed a single frond as one plant. For *D.*

acrostichoides, the number of spores per plant was calculated by counting and multiplying together the means of the number of fertile fronds per plant, the number of fertile pinnae per frond, the number of sori per pinnae, the number of sporangia per sorus, and the number of spores per sporangium. *Deparia acrostichoides* forms distinct crowns of fronds and we defined a single individual of this species as a single crown.

Spore trap experiment.—We designed this experiment to be as comparable as possible the study of Peck, Peck, and Farrar (1990) and our methods for this experiment closely follow theirs. Spore trap experiments were conducted on different individuals than those from which the spore counts were made. We mounted 75 × 25 mm clear glass microscope slides that were coated on one side with double stick cellophane tape on metal hangers made of 12-gauge steel wire bent at the tops to hold the slide in place. The height of hangers was tailored specifically for each species based on measurements of average length to the middle of the fertile frond from representative specimens of both species housed at The Ohio State University Herbarium (OS). Hanger height was 27.94 cm and 48.26 cm for *A. pedatum* and *D. acrostichoides*, respectively after a 5.08 cm offset to account for the height of the slide.

Hangers were placed at distances of 0.1, 0.5, 1, 3, and 5 m from the center of a study clump in all four cardinal directions (N, S, E, W). The tape-coated side of the slide was placed facing the clump to capture any dislodged spores. Slides were hung vertically (long side perpendicular to ground). For *A. pedatum*, a clump was defined as a group of several fertile fronds that appeared to be from a single genet. For *D. acrostichoides*, a clump was defined as a group of fronds less than one foot from each other and appearing as one genet. For all clumps of a given species, additional fertile fronds were removed to maintain equal numbers of fertile fronds between clumps. To discount the possibility of errant spores from nearby clumps becoming trapped on slides, fertile fronds of all fern species within a 10 m radius of the center of a clump were removed. Spore traps were placed around 10 clumps of each species and each spore trap was replaced once during sporulation to ensure the capture of spores at the time of release from the sporangium, as well as to maximize spore entrapment. The first set of spore trap slides was set up between 17 and 20 July 2008 and actively trapped spores until the second set of slides were put out between 29 August and 1 September 2008. The second set was taken down 16 November 2008 after all fronds had senesced. In total, these two sets produced 400 slides for each species and 80 slides for each distance from the center of the clump. The number of spores per slide was counted under a stereo microscope at 40 X magnification and the sum for both sets of each distance/direction combination was calculated.

To examine the correlation between distance and spore number for both species a nonlinear least squares model was fitted to the data using the “nls” function in R (R Core Team, 2014). Specifically, we fitted a power law to the data based on the results of previous studies of spore dispersal patterns (Okubo and Levin, 1989; Sundberg 2005) where number of spores = $a \times \text{distance}^b$. This function estimates the coefficients a and b to find the best fitting model. We

used arbitrary starting values of $a = 300$ and $b = 1$ to initially attempt to fit the model. Spore number at each distance was averaged for each of the 10 replicates of each species and these values were regressed against distance from the clump.

Evolution of spore production in ferns.—Data on spore production from multiple sources were combined with those from this study (Table 1). If no direct count was reported, we extrapolated counts of the means of individual fertile structures to obtain an estimate, just as we did when measuring spore estimates for *D. acrostichoides* above, using herbarium specimens housed at OS and WIS (Appendix 1). If multiple estimates existed for a species, these numbers were averaged. Because fertile frond production varies between species, and total number of fertile fronds is often not reported, spore production estimates were standardized to represent production per frond. For heterosporous ferns, the sporocarp is homologous with a frond of homosporous ferns (Nagalingum, Schneider, and Pryer, 2006), so estimates were taken of spore output for a single sporocarp. Total spore output for heterosporous species was regarded as the sum of megaspores and microspores. As fertile frond area may limit the number of sporangia produced and thus limit total spore output, we also recorded the fertile area of the frond as a measure of length of the frond covered by sporangia in cm multiplied by maximum width of the sporangia-covered portion of the frond. When possible, measures were taken from the same sources as spore counts. If not, measures were taken from well-pressed specimens housed at WIS.

We placed these estimates of spore production in the context of the hypothesis of fern relationships and divergence times recently proposed by the supermatrix plastid phylogeny of Testo and Sundue (2016), which contains all species for which data exist except for *Alsophila bryophila*, *Cyathea delgadii*, and *Schizaea rupestris*. For these species, we used *Alsophila cuspidata*, *Cyathea furfuracea*, and *Schizaea pusilla* as placeholder tips, respectively.

To examine if spore production in ferns is phylogenetically conserved, phylogenetic signal was examined using Blomberg's K statistic (Blomberg, Garland, and Ives, 2003) in the R package *picante* (Kembel et al., 2010) using the "phylosignal" function with 10 000 simulations. The K statistic compares the observed signal in a trait on a phylogeny to the signal under a Brownian motion model of trait evolution. A $K = 1$ indicates that the trait values of the tips are correlated equal to the Brownian expectation, whereas a $K = 0$ indicates no correlation under the Brownian expectation. The statistical significance of the K statistic is evaluated using a null model of shuffling taxa across the tips of the phylogeny.

Correlation between spore production per frond and sporangium-producing frond area was examined by fitting linear models without accounting for phylogenetic relationships using the R function "lm" and also by accounting for phylogenetic relationships using the R package *phyolm* under a Brownian motion model (Ho and Ané, 2014).

TABLE 1. Spore production estimates per frond across ferns. Note: All independent estimates are noted here. Therefore, multiple estimates may exist of a species. Vouchers for new data based on herbarium specimens are listed in Appendix 1.

| Species | Family | Spores (10 ⁶) | ln (spores) | Source |
|---|------------------|------------------------------|----------------|--|
| <i>Adiantum pedatum</i> L. | Pteridaceae | 1.8 6.5 | 7.50 8.78 | this study Peck (1980); Peck, Peck, and Farrar (1990) |
| <i>Alsophila bryophila</i> R.M. Tryon | Cyatheaceae | 320 | 12.68 | Conant (1976) |
| <i>Alsophila hornei</i> Baker | Cyatheaceae | 18.74 | 9.84 | Ash (1987) ^a |
| <i>Asplenium aureum</i> Cav. | Aspleniaceae | 10 | 9.21 | Page (1979) |
| <i>Asplenium onopteris</i> L. | Aspleniaceae | 3 | 8.01 | Page (1979) |
| <i>Asplenium rhizophyllum</i> L. | Aspleniaceae | 0.4 | 5.99 | Peck (1980); Peck, Peck, and Farrar (1990) |
| <i>Asplenium trichomanes</i> L. | Aspleniaceae | 0.75 | 6.62 | Page (1979) |
| <i>Athyrium filix-femina</i> (L.) Roth | Athyriaceae | 8.83 | 9.09 | Peck (1980) ^b ; Peck, Peck, and Farrar (1990) ^b |
| | | 20 | 9.90 | Page (1979) |
| | | 46.03 | 10.74 | Esteves and Dyer (2003) |
| <i>Azolla caroliniana</i> Willd. | Salviniaceae | 0.003 | 1.12 | Svenson (1944); Nagalingum Schneider, and Pryer (2006) |
| <i>Azolla filiculoides</i> Lam. | Salviniaceae | 0.01 | 2.16 | Svenson (1944); Nagalingum, Schneider and Pryer (2006) |
| <i>Blechnum gibbum</i> (Labill.) Mett. | Blechnaceae | 500 | 13.12 | Page (1979) |
| <i>Blechnum spicant</i> (L.) Sm. | Blechnaceae | 1.11 | 7.02 | Esteves and Dyer (2003) |
| <i>Botrychium lunaria</i> (L.) Sw. | Ophioglossaceae | 0.1 | 4.57 | this study, Bower (1923) |
| <i>Botrychium virginianum</i> (L.) Sw. | Ophioglossaceae | 32 | 10.37 | Peck (1980); Peck, Peck, and Farrar (1990) |
| <i>Cibotium glaucum</i> (Sm.) Hook. & Arn. | Cibotiaceae | 700 | 13.46 | Page (1979) ^c |
| <i>Cryptogramma stelleri</i> (S.G. Gmel.) Prantl | Pteridaceae | 0.05 | 3.99 | Peck (1980); Peck, Peck, and Farrar (1990) |
| <i>Cyathea arborea</i> (L.) Sm. | Cyatheaceae | 363.64 | 15.00 | Conant (1976, 1978) |
| <i>Cyathea delgadii</i> Sternb. | Cyatheaceae | 6000 | 15.58 | Tryon and Tryon (1982) |
| <i>Cystopteris bulbifera</i> (L.) Bernh. | Cystopteridaceae | 1.5 | 7.31 | Peck (1980); Peck, Peck, and Farrar (1990) |
| <i>Cystopteris fragilis</i> (L.) Bernh. | Cystopteridaceae | 1.5 | 7.31 | Page (1979) |
| <i>Cystopteris protrusa</i> (Weath.) Blasdell | Cystopteridaceae | 1.25 | 7.13 | Peck (1980); Peck, Peck, and Farrar (1990) |
| <i>Cystopteris tenuis</i> (Michx.) Desv. | Cystopteridaceae | 2 | 7.60 | Peck (1980); Peck, Peck, and Farrar (1990) |
| <i>Dennstaedtia punctilobula</i> (Michx.) T. Moore | Dennstaedtiaceae | 7 | 8.85 | Cody, Hall, and Crompton (1977) |
| <i>Deparia acrostichoides</i> (Sw.) M. Kato | Athyriaceae | 20.1 | 9.91 | this study |
| <i>Dicksonia antarctica</i> Labill. | Dicksoniaceae | 750 | 13.53 | Page (1979) |
| <i>Dryopteris affinis</i> (Lowe) Fraser-Jenk. | Dryopteridaceae | 19.73 | 9.89 | Esteves and Dyer (2003) |
| <i>Dryopteris carthusiana</i> (Vill.) H.P. Fuchs | Dryopteridaceae | 75 | 11.23 | Peck (1980); Peck, Peck, and Farrar (1990) |
| <i>Dryopteris dilatata</i> (Hoffm.) A. Gray | Dryopteridaceae | 13.5 52.68 | 9.51 10.87 | Page (1979) ^d Esteves and Dyer (2003) |
| <i>Dryopteris filix-mas</i> (L.) Schott | Dryopteridaceae | 49.67 | 10.81 | Esteves and Dyer (2003) |

TABLE 1. Continued.

| Species | Family | Spores (10 ⁶) | ln (spores) | Source |
|---|------------------|------------------------------|----------------|--|
| <i>Dryopteris goldiana</i> (Hook.) A. Gray | Dryopteridaceae | 81.25 | 11.31 | Peck (1980); Peck, Peck, and Farrar (1990) |
| <i>Gleichenia dicarpa</i> R. Br. | Gleicheniaceae | 1.72 | 7.45 | Bower (1923) ^e ; this study |
| <i>Hecistopteris pumila</i> (Spreng.) J. Sm. | Pteridaceae | 0.1 | 4.61 | Tryon (1986) |
| <i>Hymenophyllum dilatatum</i> Sw. | Hymenophyllaceae | 0.65 | 6.47 | Bower (1923); this study |
| <i>Hymenophyllum nephrophyllum</i> Ebihara & K.Iwats. | Hymenophyllaceae | 0.8 | 6.47 | Bower (1923); this study |
| <i>Hymenophyllum tunbrigense</i> (L.) Sm. | Hymenophyllaceae | 0.03 | 3.52 | Richards and Evans (1972) |
| <i>Hymenophyllum wilsonii</i> Hook. | Hymenophyllaceae | 0.04 | 3.78 | Richards and Evans (1972) |
| <i>Leptopteris wilkesiana</i> (Brack.) Christ | Osmundaceae | 3 | 8.01 | Ash (1986, 1987) ^a |
| <i>Lygodium circinatum</i> (Burm. f.) Sw. | Lygodiaceae | 2.07 | 7.63 | Bower (1923) ^f ; this study |
| <i>Lygodium microphyllum</i> (Cav.) R. Br. | Lygodiaceae | 0.37 | 5.92 | Volin et al. (2004); this study |
| <i>Marsilea deflexa</i> A. Braun | Marsileaceae | 0.02 | 2.88 | Johnson (1986) |
| <i>Marsilea macropoda</i> Engelm. | Marsileaceae | 0.06 | 4.03 | Johnson (1986) |
| <i>Marsilea minuta</i> L. | Marsileaceae | 0.01 | 1.94 | Johnson (1986) |
| <i>Marsilea nashii</i> Underw. | Marsileaceae | 0.02 | 2.80 | Johnson (1986) |
| <i>Marsilea oligospora</i> Goodd. | Marsileaceae | 0.03 | 3.51 | Johnson (1986) |
| <i>Marsilea quadrifolia</i> L. | Marsileaceae | 0.01 | 1.95 | Johnson (1986) |
| <i>Marsilea villosa</i> Kaulf. | Marsileaceae | 0.02 | 3.15 | Johnson (1986) |
| <i>Matteuccia struthiopteris</i> (L.) Tod. | Onocleaceae | 36.67 | 10.51 | Peck (1980); Peck, Peck, and Farrar (1990) |
| <i>Mohria caffrorum</i> (L.) Desv. | Anemiaceae | 2.26 | 7.72 | Bower (1923); this study |
| <i>Ophioglossum pendulum</i> L. | Ophioglossaceae | 1.58 | 7.36 | Bower (1923); this study |
| <i>Osmunda claytoniana</i> L. | Osmundaceae | 7.5 | 8.92 | Peck (1980); Peck, Peck, and Farrar (1990) |
| <i>Osmunda regalis</i> L. | Osmundaceae | 50 | 10.81 | Page (1979) |
| <i>Polypodium macaronesicum</i> A.E. Bobrov | Polypodiaceae | 15 | 9.62 | Page (1979) |
| <i>Polypodium virginianum</i> L. | Polypodiaceae | 0.18 | 5.16 | Peck (1980); Peck, Peck, and Farrar (1990) |
| <i>Pteridium aquilinum</i> (L.) Kuhn | Dennstaedtiaceae | 300 | 12.61 | Conway (1957) |
| <i>Schizaea rupestris</i> R. Br. | Schizaeaceae | 0.02 | 2.95 | Bartoo (1929) |
| <i>Stromatopteris moniliformis</i> Mett. | Gleicheniaceae | 0.06 | 4.10 | Bower (1923); this study |
| <i>Todea barbara</i> T. Moore | Osmundaceae | 50 | 10.82 | Page (1979) |
| <i>Vandenboschia radicans</i> (Sw.) Copel. | Hymenophyllaceae | 0.91 | 6.81 | Bower (1923); this study |
| <i>Woodsia obtusa</i> Torr. | Woodsiaceae | 10 | 9.21 | Peck (1980); Peck, Peck, and Farrar (1990) |

^a Assumes half of fronds produce spores on a fertile plant.

^b As *A. angustum* (Willd.) C. Presl.

^c As *C. splendens* (Gaudich.) Krajina.

^d As *D. austriaca* (Jacq.) Woy. ex Schinz & Thell.

^e As *Gleichenia circinnata* Sw.

^f As *L. dichotomum* (Cav.) Sw.

^g Spore count per sporangium reported from *O. falcatum* (C. Presl) Fowler; other measures from *O. pendulum*.

RESULTS

Spore production.—As the observed mean number of spores per sporangium was less than the 64 spores expected to be produced by these leptosporangiate ferns (52.0 and 53.0 for *A. pedatum* and *D. acrostichoides*, respectively), we attributed this to spore release in transit from the field to lab and we therefore assumed the theoretical 64 spores per sporangium when calculating spore production estimates. We estimated the number of spores produced by a frond of *A. pedatum* to be 2.2×10^6 (Table 2). For *D. acrostichoides*, the number of spores produced per frond was estimated to be nearly nine times greater, 20.1×10^6 (Table 3).

Spore dispersal.—Of the possible 800 slides recoverable for both species, 682 were recovered. The remaining 118 slides were lost as a result of falling of hangers and breaking, not recovered at all, or covered in detritus and therefore uncountable. In all, 288 sample/distance/direction combinations had slides from both sets recovered. Most loss of slides occurred at the greater distances from each clump. Most trapped spores were recovered within 1 m of the parent clump (Table 4). At a distance of 5 m, virtually no spores of *A. pedatum* were recovered. For *D. acrostichoides*, a low but non-zero number of spores were recovered. Based on the estimated parameters of the nonlinear regression models, both species show correlation between spore number and distance from parent plant (Fig 1.). For *A. pedatum*, the estimated parameter values, standard error, and probability of the parameter being zero were $a = 226.55 \pm 121.66$ ($p = 0.16$) and $b = -0.62 \pm 0.25$ ($p = 0.092$). For *D. acrostichoides* the parameter estimates were $a = 491.99 \pm 124.43$ ($p = 0.029$) and $b = -0.82 \pm 0.11$ ($p = 0.0056$). These results suggest that while *D. acrostichoides* releases a larger number of spores than *A. pedatum*, spores have a faster settling speed (the rate of decline, parameter b).

Evolution of spore production.—Sixty-three estimates of spore production were obtained (Table 1). Estimates were standardized to represent production per frond. After averaging conspecific reports, spore production estimates were obtained for 59 species. These 59 species represent a wide range of morphological, phylogenetic, and ecological diversity in ferns. Estimates of spore production per fertile frond are highest in tree ferns (Cyatheaales) and lowest in heterosporous ferns (*Azolla*, *Marsilea*). Spore production is both conserved within clades (*Cystopteris*, *Dryopteris*) and divergent within clades (*Asplenium*, Dennstaedtiaceae, *Hymenophyllum*, *Polypodium*) (Fig. 2). A test for phylogenetic signal indicates that spore production in ferns has less phylogenetic conservatism than expected under Brownian motion but differs significantly from 0 ($K = 0.416$, $P = 0.0001$).

Of these 59 species, an estimate of frond area covered by sporangia was obtained for 42 species. Linear regression of natural log number of spores versus frond area covered in sporangia suggests a significant correlation both without phylogenetic correction (adjusted $R^2 = 0.59$, $p = 1.63 \times 10^{-9}$) and with phylogenetic correction ($p = 1.62 \times 10^{-8}$) (Fig. 3).

TABLE 2. Spore production in *Adiantum pedatum*. Standard deviation is in parentheses after the mean. One individual was viewed as equivalent to one frond. An estimate of 64 spores per sporangium was used to calculate spore production (see results for justification).

| Spores per sporangium (n = 21) | Sporangia per indusium (n = 21) | Indusium length (mm) (n = 21) | Sporangia per mm indusium (n = 21) | Indusium length per pinna (mm) (n = 30) | Fertile pinnae per frond (n = 8) | Spore production |
|---|--|--|---|--|---|---------------------|
| 52.0 (4.6) | 56.7 (25.4) | 2.1 (0.71) | 26.2 (6.8) | 124.1 (96.5) | 10.6 (1.8) | 2.2×10^6 |

DISCUSSION

Spore production and dispersal at Deep Woods.—Estimates of spore production are both consistent and inconsistent with those of Peck, Peck, and Farrar (1990). Their estimate of spore production in *A. pedatum* was 6.5×10^6 spores per frond while ours is about one third of that estimate, 2.2×10^6 spores per frond. While no quantitative data are presented here, we attribute this discrepancy to fronds of smaller size than is typical for this species on the mesic slope studied. Studies have demonstrated that spore production between individuals at a site varies based on such factors as shade, edaphic features, and precipitation, so these might be at play here (Conway, 1957; Greer and McCarthy, 2000). For *D. acrostichoides*, the estimate of 58.3×10^6 spores per plant is in agreement with the estimate of 53×10^6 spores per plant in the closely related *Athyrium filix-femina* by Peck, Peck, and Farrar (1990). Overall, these results suggest that caution should be exercised when estimating spore production in the field, as production estimates likely vary between populations and years.

Estimates of the extent to which spores of both *A. pedatum* and *D. acrostichoides* are dispersed are highly consistent with the results of Peck, Peck, and Farrar (1990). Peck, Peck, and Farrar (1990) found that, even with the fertile portion of the frond projecting beyond the densest part of the herbaceous layer, most spores were found at distances less than 2 m from the parent plant. Dispersal past this distance was a rare occurrence. Even when spore traps were extended to 5 m, very few spores were trapped beyond this point. This appears to be a widespread trend in free-sporing plants, as similarly executed studies on bryophytes have shown most spore dispersal is within 2 m of the parent plant and often well within 1 m (McQueen, 1985;

TABLE 3. Spore production in *Deparia acrostichoides*. Standard deviation is in parentheses after the mean. One individual was viewed as equivalent to one crown. Note: an estimate of 64 spores per sporangium was used to calculate spore production (see results for justification).

| Spores per sporangium (n = 36) | Sporangia per sorus (n = 37) | Sori per pinna (n = 37) | Fertile pinnae per frond (n = 15) | Fertile fronds per plant (n = 18) | Spore production (64 spores/ sporangium) |
|---|---------------------------------------|----------------------------------|--|--|--|
| 53.0 (8.6) | 39.4 (11.7) | 210.4 (97.3) | 37.9 (9.3) | 2.9 (1.6) | 58.3×10^6 |

TABLE 4. Mean number of spores trapped at each sampled distance for *Adiantum pedatum* and *Deparia acrostichoides*.

| Species | 0.1 m | 0.5 m | 1 m | 3 m | 5 m |
|-------------------------------|---------|---------|--------|-------|-------|
| <i>Adiantum pedatum</i> | 904.55 | 639.82 | 49.19 | 1.97 | 0.067 |
| <i>Deparia acrostichoides</i> | 3226.12 | 1132.88 | 324.51 | 20.28 | 19.43 |

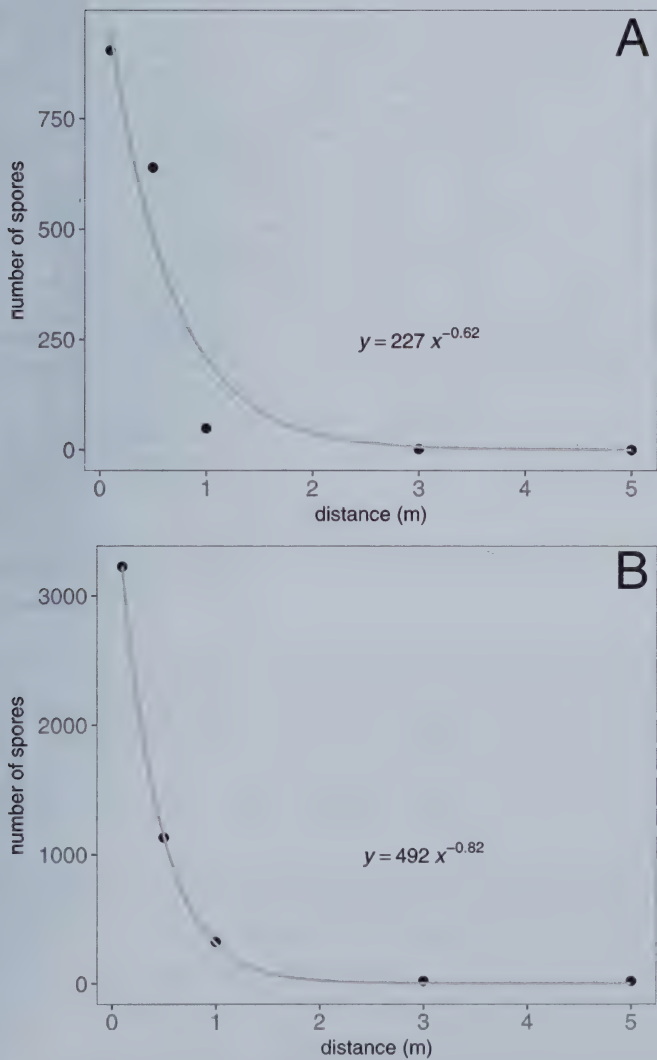


FIG. 1. Nonlinear regression of natural log of spore number versus distance from study clump for *Adiantum pedatum* (A) and *Deparia acrostichoides* (B) with accompanying best-fitting regression equations.

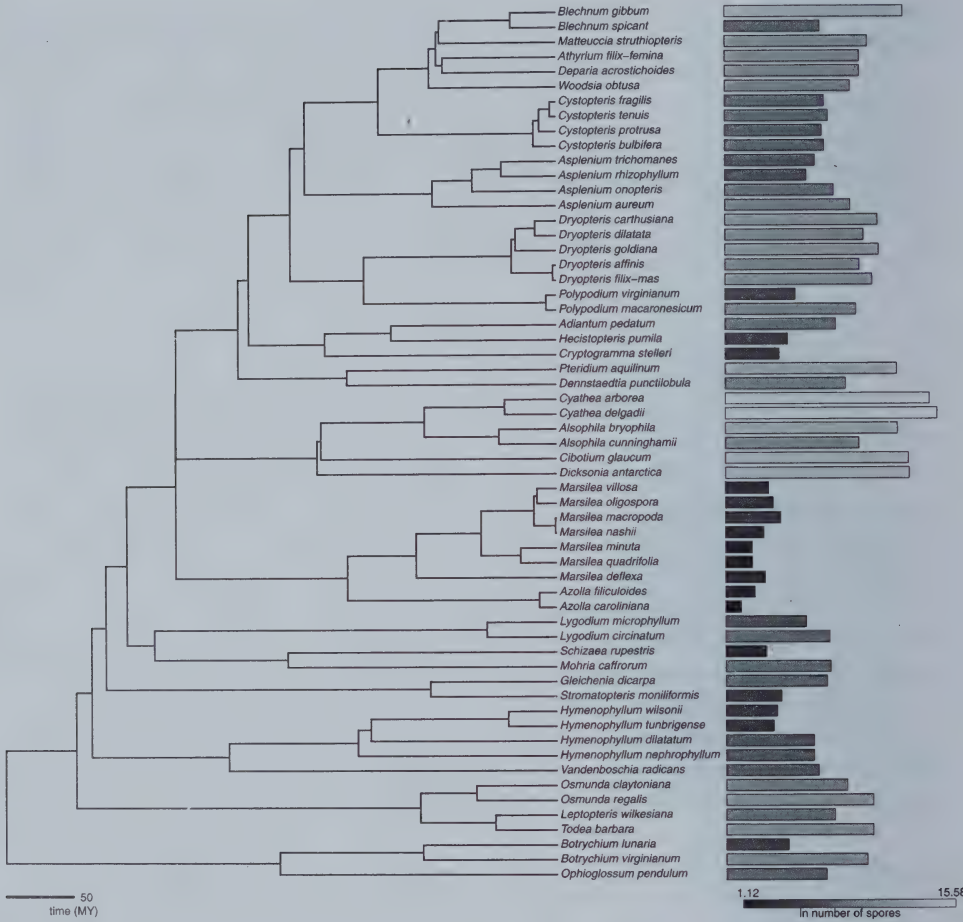


FIG. 2. Chronogram of ferns modified from Testo and Sundue (2016). Bar size and extent of shading at the right of the tips of the tree represent spore production (in natural log millions of spores), as in Table 1. Darker shading of the box indicates fewer spores produced while lighter shading of the box indicates more spores produced.

Söderström and Jonsson, 1989; Stoneburner, Lane, and Anderson, 1992; Sundberg, 2005).

Based on previous results from ferns, plant height anecdotally appears important in dispersal pattern. For example, in the upper Midwest of the U. S. A., *Botrychium virginianum* attains a median height of 30 cm (Tryon et al., 1940) and is only expected to disperse most spores approximately 3 m from the parent plant (Peck, Peck, and Farrar, 1990). Furthermore, an 8 m tall *Cyathea arborea* is expected to disperse most spores within a 7.5 m radius (Conant, 1978) and a 6 cm tall *Botrychium pumicola* is expected to disperse most spores within a 1 m radius (Roe-Andersen and Southworth, 2013). However, our results suggest that factors in addition to frond height are important in dispersal distance. The estimated value of the b parameter for *D.*

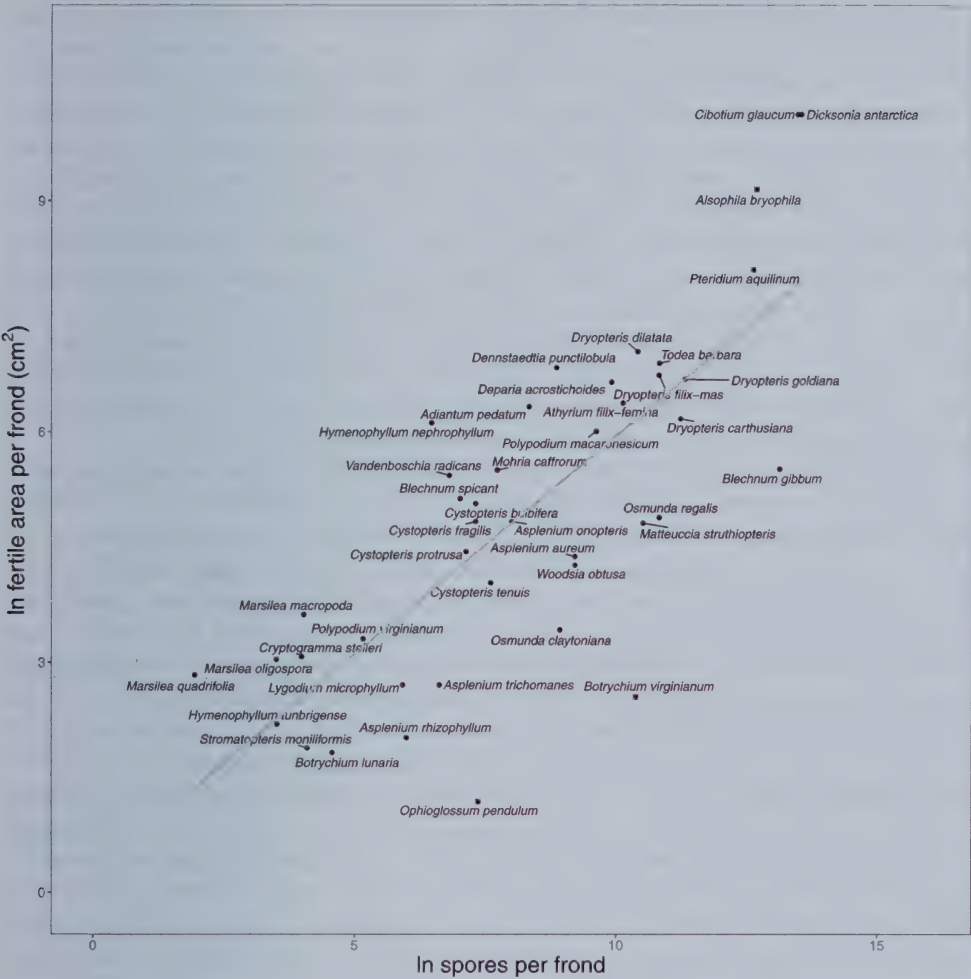


FIG. 3. Linear relationship between spores produced per frond (in natural log millions of spores) and frond area covered by sporangia (in natural log cm²). The regression line indicates the linear relationship between these variables when phylogenetic relationships are not taken into consideration, although the slope is significantly non-zero in both cases.

acrostichoides suggests that spores settle faster for this species than for *A. pedatum* despite the greater height of the spore traps and higher estimated mean fertile frond height (33.0 cm for *A. pedatum* and 53.3 cm for *D. acrostichoides*, see Materials and Methods).

Based on observation of spores trapped at identical distances but different directions, the direction of prevailing winds is an important factor in dispersal biology of ferns (data not shown). For *A. pedatum*, most spores are dispersed to the north and most study plants were located on a north-facing slope. At Deep Woods, wind likely blows down the slope from the crest of the ridge and disperses spores northward. By contrast, plants of *D. acrostichoides* were

mostly in a riparian corridor with more dense vegetation surrounding them, resulting in a more even dispersal pattern of spores. Neither wind speed nor density of understory was quantified during this study. However, the relatively dense understory layer of *D. acrostichoides* might be expected to reduce wind speeds around these plants and hinder spore dispersal. Thus, in a situation where understory vegetation was equally dense, a greater proportion of detected spores in *D. acrostichoides* might be found at larger distances from the parent plant. The effect of open habitat on dispersal is well-illustrated in the case of *Botrychium pumicola*, where 6 cm tall plants can disperse enough spores to distances exceeding 10 m to be readily detected (Roe-Andersen and Southworth, 2013). Furthermore, ferns of temperate deciduous forests may shed spores over winter (Farrar, 1976). Increased winds, especially in more open temperate forest habitats during this season increase the probability of long distance dispersal in retained spores on senesced fronds.

Spore size has often been implicated as a predictor of dispersal distance, with large-spored species capable of dispersing shorter distances than small-spored species (Sundberg, 2005; Norros et al., 2014). While not explicitly part of our model, spores of *A. pedatum* and *D. acrostichoides* differ in size and shape (33 μm ; trilete and $41 \times 25 \mu\text{m}$; monolete, respectively). In agreement with this prediction, spores of *A. pedatum* appear capable of travelling greater distances before settling. To what extent spore size and the aerodynamic benefits of shape interact with other variables should be considered in future studies.

One major drawback of this study, like that of Peck, Peck, and Farrar (1990) is that only spore dispersal laterally from the plant was measured. However, movement of spores in the air column above the plant is important (Gregory and Hirst, 1957). Upwelling of air, especially in open habitats, may elevate spores to distances where they can engage in long distance dispersal. However, Gregory and Hirst (1957) report the number of spores found above plants to be low (4 to 36 spores per cubic meter), and therefore does not contradict our principle finding of disproportionately high spore dispersal at a very local scale and the rarity of long distance dispersal in ferns.

While the probability of individual spores being transported long distances is indeed rare, the sheer number of spores produced by individual plants annually, combined with the perennial habit of most ferns combines to make long-dispersal of a small but significant number of spores highly likely. Though it remains to be investigated, shorter dispersal distances due to spore morphology or habitat preferences might be counteracted by frond height and/or spore output. Future studies should account for additional directions of spore dispersal as well as a suite of additional ecological variables as parameters in their models including herb layer density, frond height, spores size, aerodynamic properties of spores, humidity, and local wind speeds.

Evolution of spore production in ferns.—When discussing fern biogeography, citations of spore production estimates are often made. The sheer number of spores produced is often cited as a reason for widespread occurrence of species. In this survey of fern spore production estimates, we have noted discrepancies between reports of spore output for supposedly

identical species in the literature as well as inconsistencies in whether production estimates are reported for individual fronds or plants. While some of these differences may be due to ecological effects or differences in ploidy level, some differences appear irreconcilable even if some phenotypic plasticity is allowed (e.g., estimates for *Dryopteris dilatata*) (Table 1). It is clear that more systematic and thorough studies with clearly stated methods of estimation are needed, especially on *in situ* populations across the range of particular species in order to account for these potentially confounding factors. Nevertheless, the data that exist on spore production in ferns are suggestive of both phylogenetic conservatism in some clades and also sharp discontinuities between closely related species in other clades (Table 1; Fig. 2). Additionally, the presence of a relatively low number of spores produced per frond in Hymenophyllales, Ophioglossales, and Schizaeales is suggestive of low spore production in the common ancestor of ferns, though more taxonomic sampling is required before a reliable ancestral state reconstruction can be made.

Relative to other orders, Cyatheales and Polypodiales, dramatic switches to increased spore production are evident. Additionally, dramatic shifts occur within Polypodiales. These clades also tend to have large frond area. Our preliminary data examining the relationship between a simple metric of fertile frond area and spore output per frond (Fig. 3) suggests there is a strong relationship between spore output and extent of the spore producing area of the frond, even when accounting for phylogenetic non-independence. In Fig. 3, species below the regression line produce a relatively large number of spores per unit area covered by sporangia, whereas species above the regression line produce a relatively small number of spores per unit area covered by sporangia.

Not surprisingly, species with sporangia borne on fronds or portions of fronds that are densely covered by sporangia and non-photosynthetic at maturity (Ophioglossaceae, *Matteuccia*, *Osmunda*) produce more spores per unit area than those species with sporangia well-separated by photosynthetic tissue. Eusporangiate ferns in particular produce the most elevated number of spores per unit area of any of the ferns examined thus far, likely a result of the thousands of spores produced per sporangium as opposed to dozens to hundreds of spores per sporangium in other ferns. By contrast, in Salviniales, abortion of spore mother cells is likely a partial reason for low spore output, and especially low in relation to "frond" (sporocarp) area. However, in ferns as a whole, it is unclear whether natural selection is acting to increase spore output, photosynthetic area, or both. Furthermore, it is unclear to what extent sporangial density is related to reproductive and physiological factors.

Perhaps a more important metric than spore production per frond is spore production per individual. This requires additional data on the average number of fertile fronds per plant, but such data are rarely reported in the literature. We note that species producing fewer spores per frond (i.e., *Asplenium* spp.) may often compensate somewhat for lower production by producing more fertile fronds per plant than species that produce more spores per frond.

In the future, the extent to which spore production is also related to habitat should be examined. A detailed study of the microhabitat of sympatric *Cyathea arborea* and *Alsophila bryophila* by Conant (1976) showed that *C. arborea* is most abundant in disturbed habitats, whereas *A. bryophila* is more abundant in undisturbed forests. This preference for disturbed habitats in *C. arborea* (and *C. delgadii*, see Arens (2001)) is reflected in higher numbers of spores produced, nearly 40 million more per frond in *C. arborea* (Conant, 1976). If spore production is correlated with amount of habitat disturbance, a natural extension of this hypothesis is that spore production is also correlated to gametophytic traits, specifically genetic load or selfing ability. For species adapted to disturbance or other patchy habitats such as rock faces, one might expect gametophytes to be self-compatible (Peck, 1985). Preliminary evidence using phylogenetic generalized least squares regression of gametophyte isolate potential against spore production for 20 species suggests no relationship between these two variables (J. Rose, unpublished data), arguing against this hypothesis.

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APPENDIX 1

Herbarium voucher information from which new data for spore production estimates per frond were gathered.

Botrychium lunaria: anonymous 18 August 1870 (WIS); Curtis s.n. (WIS); Gillett s.n. (WIS); Griggs s.n. (OS); Hensen 3558, 1147 (WIS); Jennings s.n. (OS); Johnson 1093 (WIS); Mairlot s.n. (WIS); Moldenke 20758 (WIS); Pebphectar 446 (WIS); Potter 2170 (WIS) Thompson s.n. (WIS)

Gleichenia dicarpa: Franc 346 (WIS)

Hymenophyllum dilatatum: Doore 260, 318 (WIS); Lowden s.n. (OS); Lloyd s.n. (OS)

Hymenophyllum nephrophyllum: Dundwiddie 561 (WIS); Lowden s.n. (OS)

Lygodium circinatum: Gates 5486 (WIS); Grether 3572 (WIS); Topping s.n. (27 September 1908, 14 April 1911, 19 June 1911, 26 October 1913) (OS)

Lygodium microphyllum: Raulerson 17390 (OS)

Mohria caffrorum: Buchanan s.n. (WIS)

Ophioglossum pendulum: Ewan 327 (WIS); Forves s.n. (OS); Sohmer 6403, 6050 (WIS)

Stromatopteris moniliformis: Baumann 15438 (WIS); Foster 29 (WIS); Franc s.n. (WIS); McMillan 5138 (WIS); Musselman 5382, 5982 (WIS)

Vandenboschia radicans: Iltis 7973x (WIS); Johnson s.n. (WIS); Lind 70 (WIS); Rzedowski 10952 (WIS)

Eleven New Scaly Tree Ferns (*Cyathea*: Cyatheaceae) from Peru

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ABSTRACT.—On the basis of field and herbarium work, 11 species of *Cyathea* from Peru are described as new. *Cyathea angelica*, *C. hierbabuena*, *C. pibya*, *C. recondita*, *C. xerica*, and *C. yambrasensis* come from the headwaters of the Marañón river in northern Peru, and *C. estevesorum*, *C. lehnertii*, *C. monteagudo*, *C. rocioae*, and *C. valliciergoana* from the eastern flanks of the Andes of central to southern Peru. Most of the new species described occur in sandstone soils and may be restricted to it. They are illustrated and discussed regarding their biogeography and possible phylogenetic affinities.

KEY WORDS.—Amotape-Huancabamba, Andes, Neotropics, sandstone, South America

Tree ferns are conspicuous and abundant components of wet tropical montane forests and some southern temperate forests across the world. They attain their highest diversity between 10° north and south of the equator (Ramírez-Barahona, Luna-Vega, and Tejedo-Díez, 2011, Ramírez-Barahona, Barrera-Redondo, and Eguiarte, 2016) and in areas with steady rainfall and warm to cool temperatures throughout the year (Bystrakova, Schneider, and Coomes, 2011). Andean tropical montane forests support one of the richest tree fern floras on the planet, harboring more than 200 species, many of which occur in Peru (Lehnert, 2011). As with most ferns, they generally have a low degree of endemism (Barrington, 1993; Wolf, Schneider, and Ranker, 2001) but in the Neotropics they show unusually high species richness and concentration of narrow endemics on sandstone substrates at middle elevations in equatorial and subequatorial latitudes (Lehnert and Tejedor, 2016). In the last three decades, *Cyathea* has experienced a surge in the description of new taxa from Peru, due both to revision of herbarium material as well as to new collections from the field, mostly in relatively remote areas (e.g. Lehnert, 2003; Lehnert, 2006; Lehnert, 2008; Lehnert, 2009; Lehnert, 2011a; Lehnert, 2016; Lehnert and Tejedor, 2016; Leon and Moran, 1996; Moran, 1991; Smith, 2006). Many of the new taxa have come from ridges and plateaus lying between the eastern chains of the Andes and the Amazon basin, most of them on nutrient deficient soils over quartzite or sandstone. Here we describe 11 new tree fern species from Peru, most of which have been found in sandstone, and discuss their putative closest relatives, adaptations, and biogeography.

MATERIALS AND METHODS

Field trips in Peru were completed between March 2014 and March 2015. Comparative material was studied at the herbaria HOXA, NY, and USM and online resources were used for checking type specimens (plants.jstor.org) and historic literature (www.biodiversitylibrary.org). Terminology follows Lellinger (2002) and Lehnert (2011b). Line drawings in figure 10 A, C, D, F, and G were made by GC with pen and ink. All other drawings are by AT and were made using Adobe Photoshop.

TAXONOMIC TREATMENT

Cyathea angelica A. Tejedor & G. Calatayud, **sp. nov.** TYPE.—PERU. Amazonas: Bongará, Yambrasbamba, pared de arenisca 100 m SE del camino de San Lorenzo a Yambrasbamba, -5.78, -78.037, 2680 m, 21 July 2016, G. Calatayud 6009 (holotype: USM!, isotype: CUZ!). Figs. 1, 2 (A, B).

Diagnosis.—Plants small, with trunks 40–50 cm long, leaves pinnate-pinnatifid to bipinnate-pinnatifid, sori exindusiate. Unique among Neotropical *Cyathea* by markedly vertical fronds with ascending apices, and concolorous pale stramineous, translucent, fimbriate scales.

Trunk horizontal to pendent, 40–50 cm long, 3 cm diam., covered with old petiole bases; apices hidden between petiole bases; without adventitious buds. Fronds to 35 cm long, erect, unfolding subsequently. Petioles 10–13 cm long, sparsely verrucate, pale olive-green, densely hairy. Petiole scales ovate long acuminate, 6–10 × 2–3 mm, concolorous translucent pale stramineous, with fimbriate margins; scales covering the crosciers basally pale orange-brown, lanceolate with sinuous tips. Laminae to 31 × 15 cm, bipinnate-pinnatifid, firm herbaceous, opaque, bright-green; apices gradually reduced. Rachises inermous to weakly verrucate, pale olive-green. Pinnae to 8 cm long, sessile, patent, alternate, non-alate, distal segments simply adnate before ending in a pinnatifid apical section; basal pairs longer than the medial pinnae, notably reflexed. Costae 0.9–1.2 mm wide, inermous, olive-green and dark brown in dry material adaxially and abaxially. Largest pinnules 15–17 × 5–6 mm, sessile, inarticulate, 6–7 mm between the costules, oblong to oblanceolate, truncate basally, obtuse to rounded apically; costules olive-green adaxially and abaxially, brown in dry material. Segments 7.0–13.0 × 3.0–4.5 mm, sessile, adnate, and connected by laminar tissue, patent, tips rounded, proximal segments opposite basally, longer than following segments; sinuses acute, to 0.2 mm wide; segment margins entire, markedly involute; veins planar on both sides, olive-green and dark brown in dry material; sterile and fertile veins simple. Sori 0.8–1.0 mm diam., submarginal (appearing marginal if segment margins are involute), on the back of veins, mature rich orange-brown; exindusiate; receptacles globose to weakly columnar, 0.2–0.3 mm diam., paraphyses few, stiff, hyaline, tan to brown, shorter than the sporangia (0.3–0.4 mm long). Spores not examined. Hairs and scales: rachis and costa with

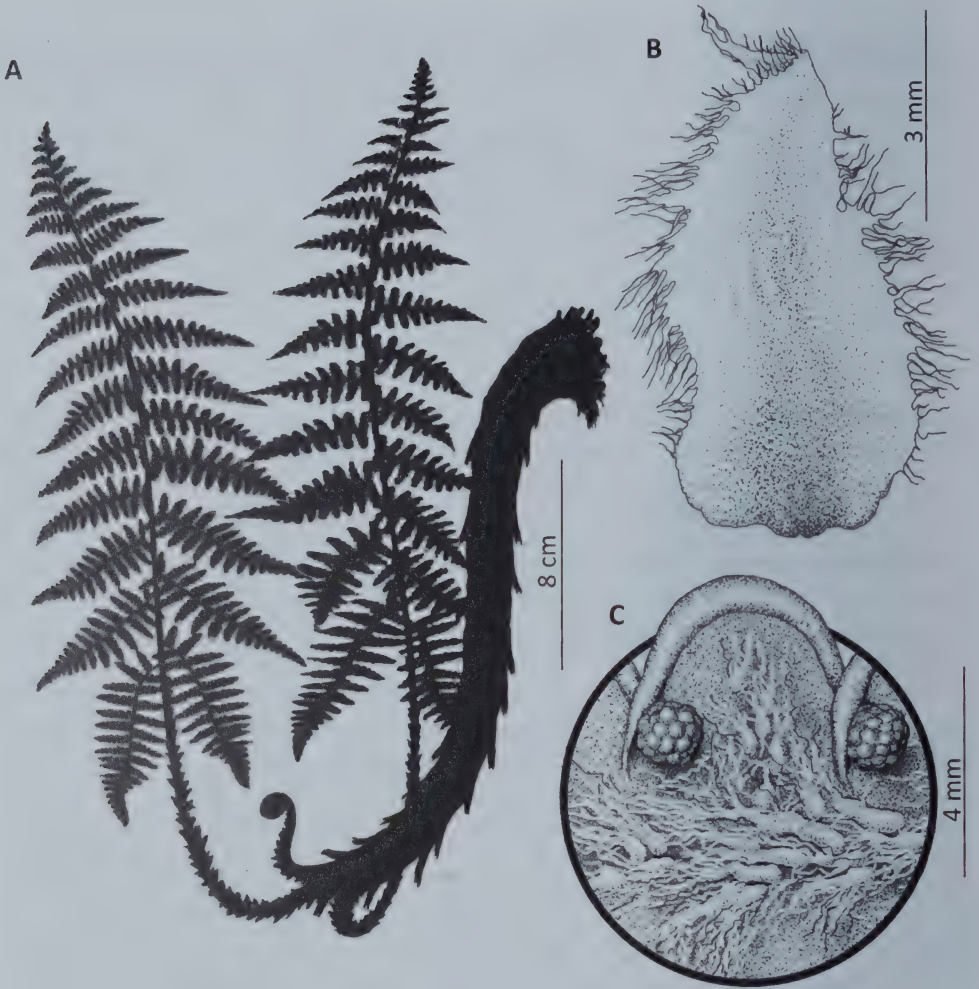


FIG. 1. *Cyathea angelica*. A. habit. B. scale. C. fertile segment abaxially. (from holotype: G. Calatayud 5609 USM)

abundant pale stramineous multicellular hairs 1.0–1.5 mm long both adaxially and abaxially; costules densely hairy with tan to white, multicellular hairs to 1.0 mm long, with many tan squamules, sub-bullate to flat 0.8–1.0 mm long, flat lanceolate ones to 3×2 mm, with fimbriate margins and hair-like linear tips; veins adaxially glabrous except for some multicellular hairs to 0.5 mm long on the midveins, abaxially midveins with a few pure white to light brown squamules, mostly bullate ones to 1.0×0.5 mm, hairs absent, rarely with trichomidia or scattered white hairs to 0.5 mm long.

Etymology.—Named after the species ascending fronds, often only two per plant, held upright like the wings of an angel.



FIG. 2. Field characters of ten newly described *Cyathea*. A. *Cyathea angelica*, fertile pinnule. B. *Cyathea angelica*, petiole. C. *Cyathea estevesorum*, habit. D. *Cyathea estevesorum*, trunk apex. E. *Cyathea hierbabuena*, petiole. F. *Cyathea hierbabuena*, habit. G. *Cyathea hierbabuena*, rachis adaxially. H. *Cyathea lehnertii*, trunk apex. I. *Cyathea monteagudo*, trunk apex. J. *Cyathea pibya*, trunk apex. K. *Cyathea rocioae*, habit. L. *Cyathea rocioae*, petiole. M. *Cyathea valliciergoana*, habit, foreground, in comparison with *Cyathea chontilla*, in the back. N. *Cyathea valliciergoana*, juvenile habit, left, in comparison with *Cyathea chontilla*, at right. O. *Cyathea valliciergoana*, trunk apex. P. *Cyathea xerica*, clump in habitat. Q. *Cyathea yambrasensis*, habit. R. *Cyathea yambrasensis*, petiole. All photos by A. Tejedor.



FIG. 3. Geographic distribution of 11 new tree ferns from Peru. Grey shading represents elevations between 50 and 3600 m.

Distribution and habitat.—Peru, known only from the type locality, a sandstone cliff in Cordillera de Colán at 2680 m (Fig. 3), where it grows on vertical surfaces or overhangs among dense mats of creeping bromeliads of the genus *Guzmania*. Its horizontal to pendular trunk and erect fronds appear to be adaptations to piercing through the curtain of creeping plants that cover the overhangs and maximizing access to light. Albeit difficult to access, sandstone cliffs are widespread across southern Cordillera Colán, therefore it is reasonable to expect that the species' range should be much more extensive than observed.

There are several other small *Cyathea* species in the Amotape-Huancabamba Gap zone (Weigend, 2004) that appear to be specialized or preferential cliff-dwellers, including *Cyathea aterrima*, (Hook.) Domin *Cyathea brucei*, Lehnert *Cyathea concordia*, B. León & R.C. Moran, *Cyathea gracilis*, Griseb. *Cyathea latevagans* (Baker) Domin, *Cyathea palaciosii* R.C. Moran, and *Cyathea thelypteroides* A.R. Sm. but they occur at lower elevations (< 2000 m) and seem to have different morphological adaptations to this habitat. Among them, *Cyathea angelica* is unique in its combination of stiffly ascending fronds and fimbriate scales. All other cliff-dwelling species have either patent, arching, or pendent fronds, and entire scales. Because of its long, lanceolate frond, *Cyathea angelica* is superficially most similar, especially seen at a distance, to the pinnate-pinnatifid species *Cyathea palaciosii*, *Cyathea thelypteroides*, and *Cyathea oreopteroides* Lehnert & A. Tejedor (not a preferential cliff-dweller, but rather specialized on wet scrub and grassland over sandy soils). In addition to the traits mentioned above, *C. angelica* differs from these species in its tendency to become bipinnate-pinnatifid as it attains larger sizes, in the densely woolly lamina abaxially, and in its submarginal sori vs. lamina glabrous to tomentous abaxially, and the medial sori in the former three. *Cyathea angelica* could also be confused from afar with *C. aterrima*, with which it might be most closely related on account of its hairiness and bipinnate-pinnatifid division, but *C. aterrima* has long lanceolate to linear scales, and arching to drooping fronds vs. ovate scales with acute tips and stiffly ascending fronds in *C. angelica*.

Cyathea estevesorum A. Tejedor & G. Calatayud **sp. nov.** TYPE.—PERU. Huánuco: Yuyapichis, debajo del Mirador camino al Cerro del Sira, - 9.4344, -74.7570, 1210 m, 8 May 2014, A. Monteagudo 25443 (holotype: HOXA!, isotype: USM!). (Figs. 2C, D, 4).

Diagnosis.—An exindusiate *Cyathea* with a bipinnate-pinnatifid lamina that is similar to the sympatric *Cyathea traillii* (Baker) Domin but differs in having broad, stiff, ovate scales with erose margins and short-stalked pinnules, rather than lanceolate, tortuous scales and long-stalked pinnules.

Tree fern, trunk to 3 m tall, erect, without old petiole bases, to 7 cm diam., covered in scales thinner than those on the petioles and croziers; frond scars visible and slightly remote from each other; adventitious buds absent. Fronds to 170 cm long, arranged in a fascicle around trunk apex, arching on ascending to patent petioles. Petioles to 40 cm long, short tuberculate, green adaxially and dark brown abaxially when older, pneumathodes narrow light-green slits ca. 20 × 2 mm; petioles scales dense near the petiole bases, soon caduceous on rachis in fully expanded fronds but persistent on petiole bases, ovate, to 15 × 4 mm, with acute tips, bicolourous with chestnut brown center and slightly paler margins, not sharply contrasted, margins stiff, with erose teeth. Laminae to 130 cm long, ovate, bipinnate-pinnatifid to pinnatisect, with abruptly reduced apex, shiny medium-dark green adaxially (dark olive-brown when dried), paler shiny green abaxially, with ca. 8 pinna pairs, alternate, basal ones

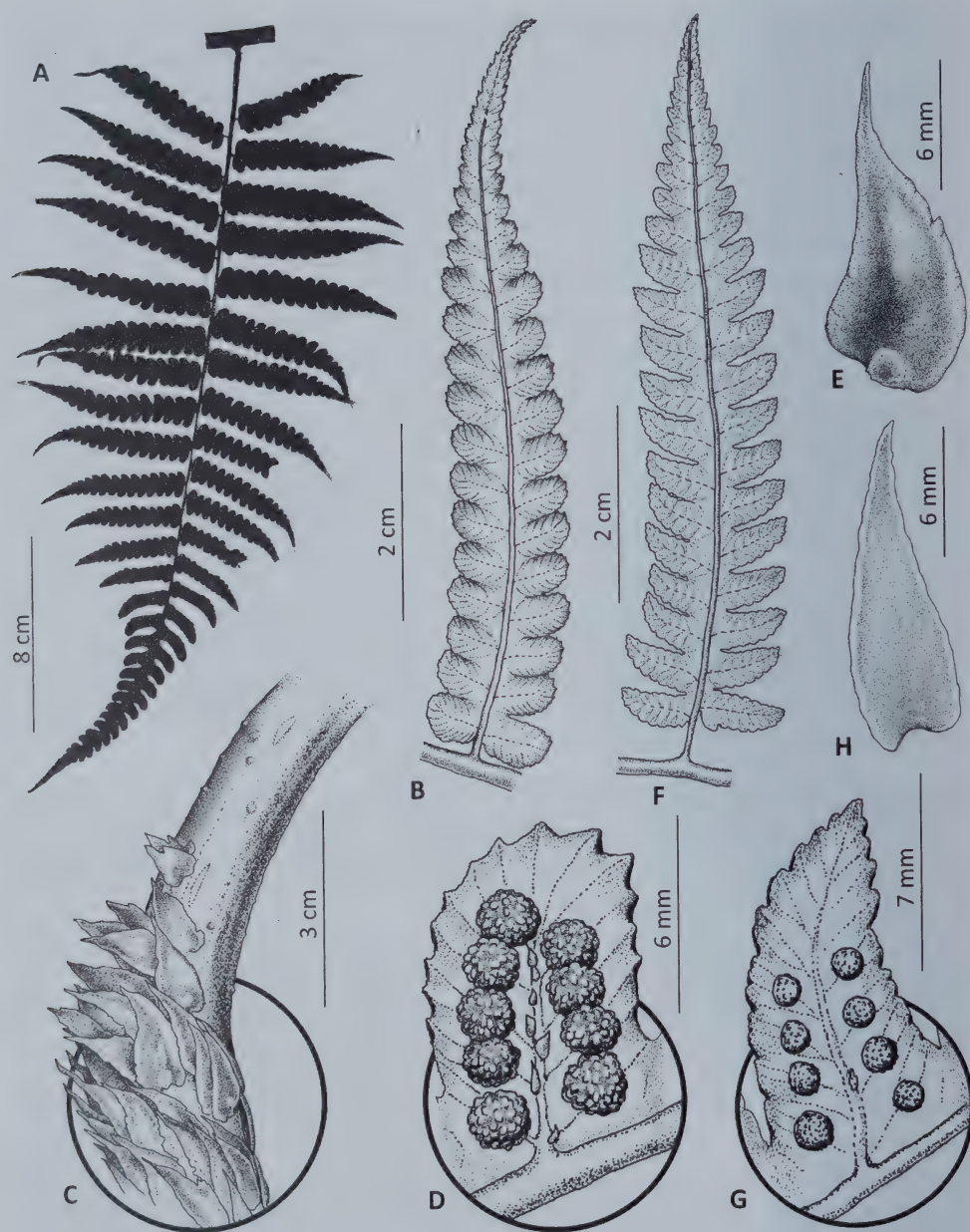


FIG. 4. A–E, *Cyathea estevesorum* (from holotype A. Monteagudo 25443 HOXA). A. fertile pinna, silhouette. B. sterile pinnules, abaxially. C. petiole. D. fertile segment, abaxially. E. petiole scale. F.G. *Cyathea trailli* (from field photograph by AT) F. sterile pinnule abaxially. G. fertile segment, abaxially. H. *Cyathea diabolica*, petiole scale (from field photograph by AT).

slightly reflexed, shorter than longest central pinnae. Frond axes inermous, dark green; costae narrowly green alate between the pinnules in distal half. Longest pinnae 35×14 cm, triangular, long stalked. Largest pinnules to 75×14 mm, narrowly triangular with acuminate tips, short stalked, pinnatisect; segments to 7×5 mm, oblong with round tips, the margins strongly serrate, veins strongly raised abaxially, flat to weakly raised adaxially, fertile veins mostly forked, rarely simple. Sori medial, to 1.2 mm diam., each with ca. 15–20 sporangia, rich brown in fresh material, indusia absent, paraphyses abundant, tortuous, nearly as long as sporangia, circa 0.4 mm long, hyaline to pale brown. Spores pale brown. Hairs and scales: Rachis abaxially with light tan scurf, denser on croziers, costules and midveins abaxially with orange-brown bullate squamules $0.3\text{--}0.5 \times 0.3$ mm.

Etymology.—Named after the Esteves siblings, Antonio (Toño) and Maria Teresa (Maite), residents of Yuyapichis and hosts and logistic leaders of numerous scientific expeditions to the Cerros del Sira.

Distribution and habitat.—*Cyathea estevesorum* is known from a small population scattered along a 2 km stretch of trail leading to the summit of Cerros del Sira, growing on humus-rich soils over well-drained sandstone ridges in lower mountain wet forest at 1200–1400 m (Fig. 3).

The general aspect of the habit of *Cyathea estevesorum* is very similar to that of *C. macrosora* (Baker ex Thurn) Domin and allies (in Peru *C. traillii*, *C. rufescens*, (Mett. ex Kuhn) Domin and *C. vaupensis* (P.G. Windisch) Lehnert), all of which share a chartaceous, shiny texture, slightly remote pinnae, and large triangular pinnules with strongly serrate segment margins. *Cyathea estevesorum* differs from these species in having short stalked pinnules (vs. long-stalked in *C. macrosora* and allies, Fig. 4 F), obtuse segment tips (vs. acute, Fig. 4 G), darker green laminae (vs. paler green), narrowly green alate costae in distal half (vs. costae non alate), and, especially, in the ovate, stiff, appressed petiole scales (vs. lanceolate, soft, tortuous, spreading scales).

Cyathea estevesorum has a stature and scales similar to those of *C. lasiosora* (Mett. ex Kuhn) Domin and *C. calamitatis* Lehnert, from which it can be distinguished by its obtuse segments with round tips and strongly serrate margins, and by its chartaceous texture vs. acute segments with crenulate margins and softer laminae in *C. lasiosora* and *C. calamitatis*. In addition, *C. calamitatis* has a dense cover of golden-brown hairs on the axes distally, whereas *C. estevesorum* is glabrous. *Cyathea lechleri* Mett., a species that co-occurs in the field with *C. estevesorum*, has similar scales and petiole scurf, but its axes are rich dark brown adaxially, its pinnules short and oblong, and has a gradually reduced frond apex, versus axes dark green, pinnules long triangular, and apex abruptly reduced in *C. estevesorum*. *Cyathea diabolica* Lehnert, is an allopatric species known from Ecuador with a superficial resemblance to *C. estevesorum*, but can be distinguished from it by its oblong pinnae, entire segment margins and fragile, translucent reddish-brown petiole scales (Fig. 4 H) vs. pinnae elliptic, segment margins serrate, and petiole scales tough, opaque dull brown in *C. estevesorum*. *Cyathea ulei* (H. Christ) Domin which co-occurs in Cerros del Sira with *C. estevesorum*, is another potentially

confusable species with bicolorous brown scales, somewhat similar to those of *C. estevesorum*. *Cyathea ulei*, however, differs in its longer pinnule stalks, to 2/3 the width of basal segments, more weakly divided pinnule, with sinuses to 1/3 the length of segments, and entire segments vs. short pinnule stalks < 1/3 the width of basal segments, deeper sinuses to 2/3 the length of segments, and serrate segments in *C. estevesorum*.

Cyathea hierbabuena A. Tejedor & G. Calatayud, **sp. nov.** TYPE.—PERU. Amazonas: Corosha, Copal, -5.793, -77.829, 2420 m, 24 July 2014, G. Calatayud 6019 (holotype: USM!, isotype CUZ!). Figs. 2E–G, 5.

Diagnosis.—An indusiate *Cyathea* that is similar to *Cyathea herzogii* Rosenst. but differs in its elongate, drooping frond apex, softer, lanceolate, discordantly bicolorous scales with dark brown to blackish centers and orange-brown margins, linear squamules along rachis adaxially, abundant, sub-bullate squamules on lamina abaxially, glabrescent veins abaxially, and smaller stature.

Tree fern, trunk to 2 m tall, erect, with old petiole bases, to 7 cm diam.; epidermis dark brown, inconspicuous; frond scars usually not visible; adventitious buds absent. Fronds to 110 cm long, arranged in a fascicle around trunk apex, patent to ascending. Petioles to 26 cm long, muricate, green to orange-brown adaxially when older, with moderately thick scurf of light golden brown, arachnoid squamules, pneumathodes narrow pale stramineous slits ca. $10\text{--}12 \times 0.8\text{--}1.0$ mm, visible among scales; petiole scales dense near the petiole bases, thinning towards rachis, long-lasting, lanceolate, to 17×4 mm, with tortuous tips, discordantly bicolorous, dark brown apically, grading to chestnut basally, with rich golden to orange-brown margins, margins rather soft, without teeth or cilia. Laminae to 80×60 cm, ovate, bipinnate-pinnatifid, with long, rather drooping, gradually reduced apex, matte light green adaxially (olive-when dried), paler matte grayish green abaxially, with ca. 11 pinna pairs, \pm alternate, rarely subopposite, basal ones strongly reflexed, much shorter than longest central pinnae. Frond axes inermous, pale brown to yellow; costae non alate. Longest pinnae 18×5 cm, long lanceolate, short stalked to 4 mm. Largest pinnules to 25×9 mm, oblanceolate with acute to elongate tips, sessile; segments to 5×2 mm, adnate, weakly ascending, oblong with rounded to obtuse tips, the margins weakly crenate, veins flat to weakly raised abaxially, flat adaxially, fertile veins mostly simple, rarely forked. Sori medial, to ca. 0.7 mm diam., each with ca. 30–40 sporangia, matte brown in fresh material, indusia sphaeropteroid, thin, with apical umbo, receptacles globose, ca. 0.2 mm diam., paraphyses few, shorter than sporangia, < 0.2 mm long, whitish. Spores not examined. Hairs and scales: Frond axes with thick, pale stramineous retrorsely curved to tortuous hairs to 1 mm and sparse, linear, light brown scales to 15 mm on adaxial side; sparse, thin, translucent, 0.5 mm long hairs on abaxial side of costae, costules, and rachis distally and adaxially on lamina along veins; veins abaxially with orange-brown sub-bullate squamules $0.5\text{--}1.0 \times 0.5$ mm.



FIG. 5. *Cyathea hierbabuena*. A. petiole scale. B. pinna, silhouette. C. fertile segment, abaxially (from G. Calatayud 5619 USM)

Etymology.—Named after the community-run Hierbabuena-Allpayacu conservation area, which protects the largest known population of the species. The name is a noun in apposition.

Paratype.—PERU. Amazonas: Bongará, Yambrasbamba, matorral sobre arenisca en el camino de San Lorenzo a Yambrasbamba, -5.771073, -78.034246, 2700 m, 24 July 2014, G. Calatayud 6068 (USM!).

Distribution, habitat, and phylogenetic affinities.—*Cyathea hierbabuena* has been found at two localities, the access trail to the Hierbabuena-Allpayacu conservation area and Cordillera de Colán, growing under scrubby forest 4–5 m tall on white sandstone (Fig. 3). At the first locality, it was found along the access trail, as the dominant tree fern in the shade of tree islands surrounded by open scrub and pastures of Eriocaulaceae.

Cyathea hierbabuena is extraordinarily similar to the allopatric species *C. herzogii* Rosenst., distributed from central Peru to Bolivia at 2000–3000 m. From afar, however, it can be distinguished from that species by its elongate, drooping frond apices, pale brown rachises adaxially, and overall smaller size, with trunks to 2 m tall and fronds to 1.1 m (frond apices acute, stiff, axes pale green to yellowish adaxially, trunks to 5 m, and fronds to 1.7 m in *Cyathea herzogii*). On close inspection, *C. hierbabuena* can also be distinguished from *C. herzogii* by its lanceolate, discordantly bicolorous scales with a dark brown central stripe and rich orange-brown margins, and with sinuous to twisted tips, by its adaxial indument on the axes composed of dense, matted light brown hairs, interspersed with numerous linear light brown scales to 15 mm long, by its glabrescent veins abaxially, and by its numerous sub-bullate scales on the abaxial side of the lamina (vs. scales nearly concolorous orange-brown, ovate with elongate stiff tips, axes abaxially without linear scales, veins abaxially with hairs, sub-bullate scales mostly lacking in *C. herzogii*).

Because of its well-developed tomentum on the lamina adaxially and croziers, grayish green fronds, and sometimes opposite pinnae, *Cyathea hierbabuena* can be mistaken for the sympatric species *Cyathea obnoxia* Lehnert. It differs from this species however in its bicolorous scales, and scurfy petiole bases (scales concolorous orange-brown and petiole bases hairy in *C. obnoxia*). With the also sympatric *C. squamipes* H. Karst. it shares the bicolorous scales and scurfy petioles, but differs in its broad orange-brown scale margins (narrow and pale brown in *C. squamipes*), shorter, more oblong pinnules and overall smaller size.

Cyathea lehnertii A. Tejedor & G. Calatayud, **sp. nov.** TYPE.—PERU. Pasco: Oxapampa, trocha al Abra La Esperanza, -10.545, -75.354, 2670 m, 12 October 2014, A. Monteagudo 27819-A (holotype: HOXA!, isotypes: CUZ!, USM!). (Figs. 2H, 6.)

Diagnosis.—An indusiate *Cyathea* with bipinnate-pinnatifid laminae, similar to *C. holdridgeana* but different in having patent fronds, and discordantly bicolorous scales with light brown centers, pale stramineous margins, and long acute apex.

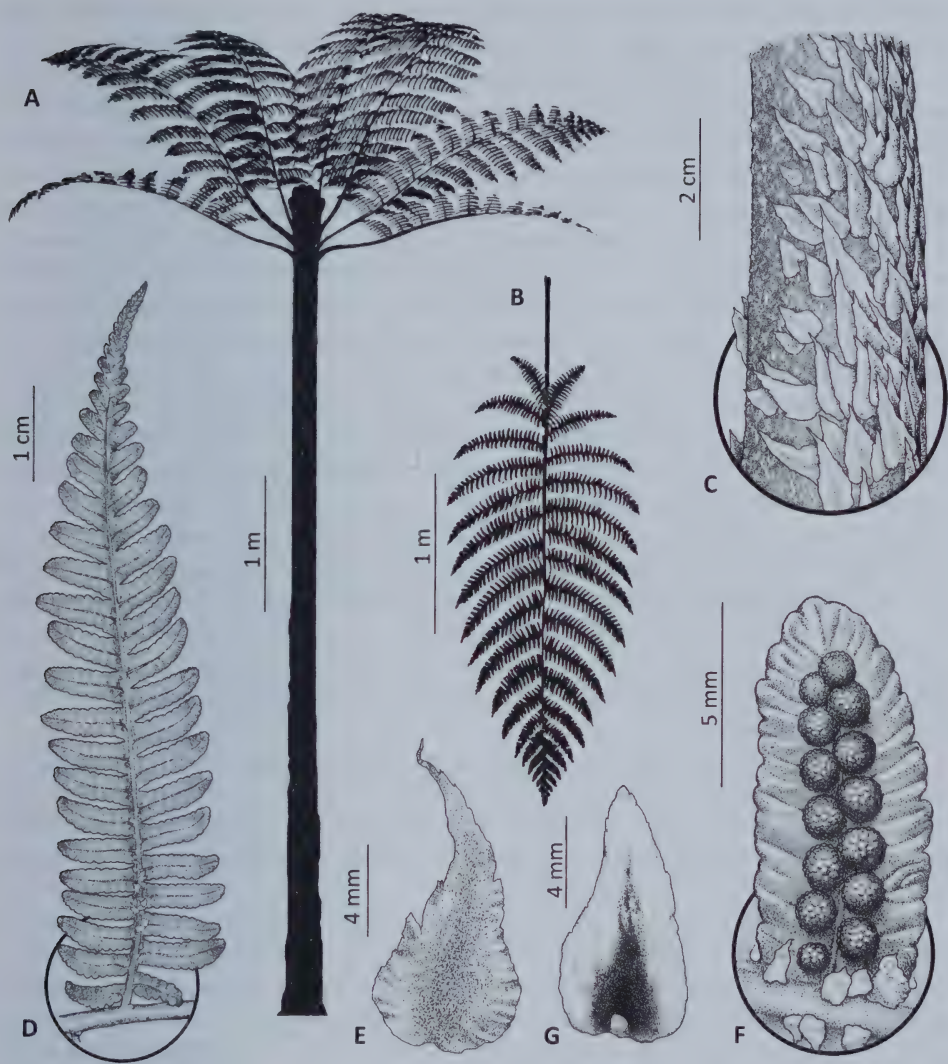


FIG. 6. A-F, *Cyathea lehnertii*. A. habit, silhouette. B. frond, silhouette. C. petiole. D. infertile pinnule abaxially. E. petiole scale. F. fertile pinnule abaxially. A, B from photographs by a. Tejedor; C-F from A. Monteagudo 27819-A HOXA. G. *Cyathea holdridgeana*, petiole scale (from field photograph by A. Tejedor).

Tree fern, trunk erect and columnar, to 5 m tall and 17 cm in diam., protuberant apex not hidden by the bases of the petioles. Fronds patent, tips gently arching, to 4 m long, arranged in a loose spiral around trunk apex; petioles to 1 m long, verrucate to muricate, dull brown, covered in dense and persistent pale stramineous scurf, formed by irregular, matted arachnoid squamules, interspersed with flat, ovate squamules with erose margins, extending along petiole and rachis. Petioles scales to 2×0.9 cm, pale

stramineous, discordantly bicolorous with light brown centers and pale stramineous margins, ovate, apex long acute, margins erose, petioles scales dense on abaxial side from the base of the petiole to half the length of the rachis. Laminae bipinnate-pinnatisect, 3×1.2 m, ovate-lanceolate, apex gradually reduced, with 16–18 pinnae pairs; basal pinnae shorter than medial pinnae and markedly reflexed; pinnae alternate, olive-green adaxially. Frond axes sparsely verrucate, dark green adaxially, brown abaxially; costae basally with large elliptic pneumathodes, ca. 9×5 mm, dark brown, flat, costae not green-alate. Longest pinnae 65×18 cm, lanceolate, stalked to 2.5 cm; costules basally with large pneumathodes to ca. 3.5×2 mm, yellowish green and markedly swollen. Largest pinnules to 9×2 cm, alternate, lanceolate, apex long acute, slightly falcate, bases truncate, sinuses narrow, about 1/5 of width of segments. Segments to $7\text{--}9 \times 3$ mm, margins crenate, fertile veins forked. Sori proximal, to ca. 1 mm; indusium hemitelioid, inconspicuous, covering 1/5 of sorus, mostly obscured by sporangia, translucent, fragile and easily lost in ruptured sori; receptacles globose; sporangia ca. 25–30; paraphyses few, shorter than sporangia. Spores pale yellow. Hairs and scales: frond axes abaxially and lamina glabrous to glabrescent on both sides, costules adaxially covered in stiff, thick, antrorsely appressed, translucent yellow, tortuous hairs ca. 2 mm long, abaxially with numerous flat, pale stramineous, lanceolate to ovate squamules ca. 0.5–3 mm; frond axes covered in pale stramineous scurf, denser abaxially, formed by small (<0.5 m), irregularly shaped, matted arachnoid squamules.

Etymology.—The specific epithet honors Marcus Lehnert, whose sweeping revisions of Neotropical tree ferns and prolific description of new species have revolutionized our understanding of this group in the New World. This is the second tree fern named after Marcus Lehnert and the first in the genus *Cyathea*.

Distribution and habitat.—*Cyathea lehnertii* grows in the understory of tall montane forest at 2500–2600 m, its patent fronds spreading under the forest canopy. It is known thus far from only three individuals, each found on separate localities in a single mountain range, Cordillera Yanachaga, of central Peru (Fig. 3). Knowledge of the species at two of the localities is based on photographs taken by AT, with only one locality represented by a herbarium voucher. Given the intense collection efforts in this area by the branch of the Missouri Botanical Garden based in Oxapampa, led by Rodolfo Vasquez, and the large size and conspicuousness of this species, the fact that this taxon is represented by a single collection and only two additional observations suggests that it is genuinely uncommon, perhaps one of the rarest of Andean tree ferns. In contrast to closely related congeners (see below), which tend to be more abundant in young to medium old successional vegetation (Lehnert and Tejedor, 2016), this species has been found so far only in mature forest.

Because of its protruding apex and dense pale stramineous indument, *Cyathea lehnertii* can be mistaken from afar for a species of the genus *Alsophila*, particularly *A. engelii* R.M. Tryon or *A. minervae* Lehnert (Fig. 2 H), but can be distinguished from those species at close range by the broad, ovate

scales, which are narrowly linear in *Alsophila*. *Cyathea lehnertii* seems to be closely related to members of the *Cyathea vilhelmii* Domin group of Neotropical *Cyathea* (including *C. abrapatriciana* Lehnert & A. Tejedor, *C. arnecornelii* Lehnert, *C. holdridgeana* Nisman & L.D. Gómez, *C. moranii* Lehnert, *C. multisegmenta* R.M. Tryon, and *C. vilhelmii*), with which it shares a gradually reduced frond apex, lack of petiole spines, and a fast growing, protruding trunk apex, not hidden by petiole bases. In this group, the species most similar to *C. lehnertii* in size, trunk morphology and frond division are *C. moranii*, *C. arnecornelii*, and *C. holdridgeana*. From *C. moranii* and *C. arnecornelii* it can be readily distinguished by the dense pale stramineous scurf and the overall pale stramineous, weakly bicolorous petiole scales with long acute apex (Fig. 6 E) vs. scurf thin and dark brown, and scales brown and markedly bicolorous with short acute apex in *C. moranii* and *C. arnecornelii*. From *C. holdridgeana*, the only other species in this group that shows dense, whitish to pale stramineous scurf on frond axes, *C. lehnertii* can be distinguished by its patent fronds and weakly bicolorous scales with light brown centers and pale stramineous margins vs. fronds markedly arching and distally drooping, and scales strongly bicolorous with dark brown to black centers and nearly pure white margins in *C. holdridgeana* (Fig. 6G).

Cyathea monteagudoii A. Tejedor & G. Calatayud, **sp. nov.** TYPE—PERU.

Pasco. Oxapampa: Chacos, carretera al cerro antena al inicio de trocha hacia Villa Rica, -10.6365, -75.287, 2600 m, 19 March 2014, A. Monteagudo 24166 (holotype: HOXA!, isotypes: CUZ!, USM!). (Figs. 2I, 7.)

Diagnosis.—A medium sized indusiate *Cyathea*, with bipinnate-pinnatisect fronds, somewhat resembling *Cyathea ruiziana* Klotzsch but differing in its smaller stature, ovate fronds, smaller number of fronds, stalked pinnules, strongly bicolored scales, and tan scurf.

Tree fern, trunk to 3 m tall, erect, stout, to 12 cm diam., without lateral buds. Fronds to 3.3 m long, patent to slightly arching, lamina to 200×110 cm, broadly ovate, apex gradually reduced, bipinnate-pinnatisect, dark brown adaxially, lighter brown abaxially when dried, dull green in live individuals; frond axes inermous, dull brown when dried. Petioles to 130 cm long, dark brown when dried, armed with stout, sharp spines; petiole scales 20×5 mm covering petiole base on adaxial side, extending sparsely along rachis, lanceolate to falcate, firm, strongly bicolorous with rich chestnut brown centers and pale brown to cream margins, differentiated margins broad, erose, tips caudate, strongly falcate or bent. Pinnae 13–14 alternate, patent to slightly arching, basal ones reflexed. Longest pinnae 55×25 cm, long stalked to 7 cm, oblong to lanceolate, apex acute, costae non-alate. Largest pinnules to 13×2.5 cm, lanceolate, stalked to 2 mm, with truncate bases, tips acute; segments to 14×5 mm, basal sinuses relatively broad, margins crenulate, veins weakly raised abaxially, flat adaxially, fertile veins forked. Sori proximal, to ca. 1.2 mm diam., each with ca. 50 sporangia, light brown in dried material, indusia sphaeropteroid, thin, with an umbo, dull brown when dry, paraphyses few,

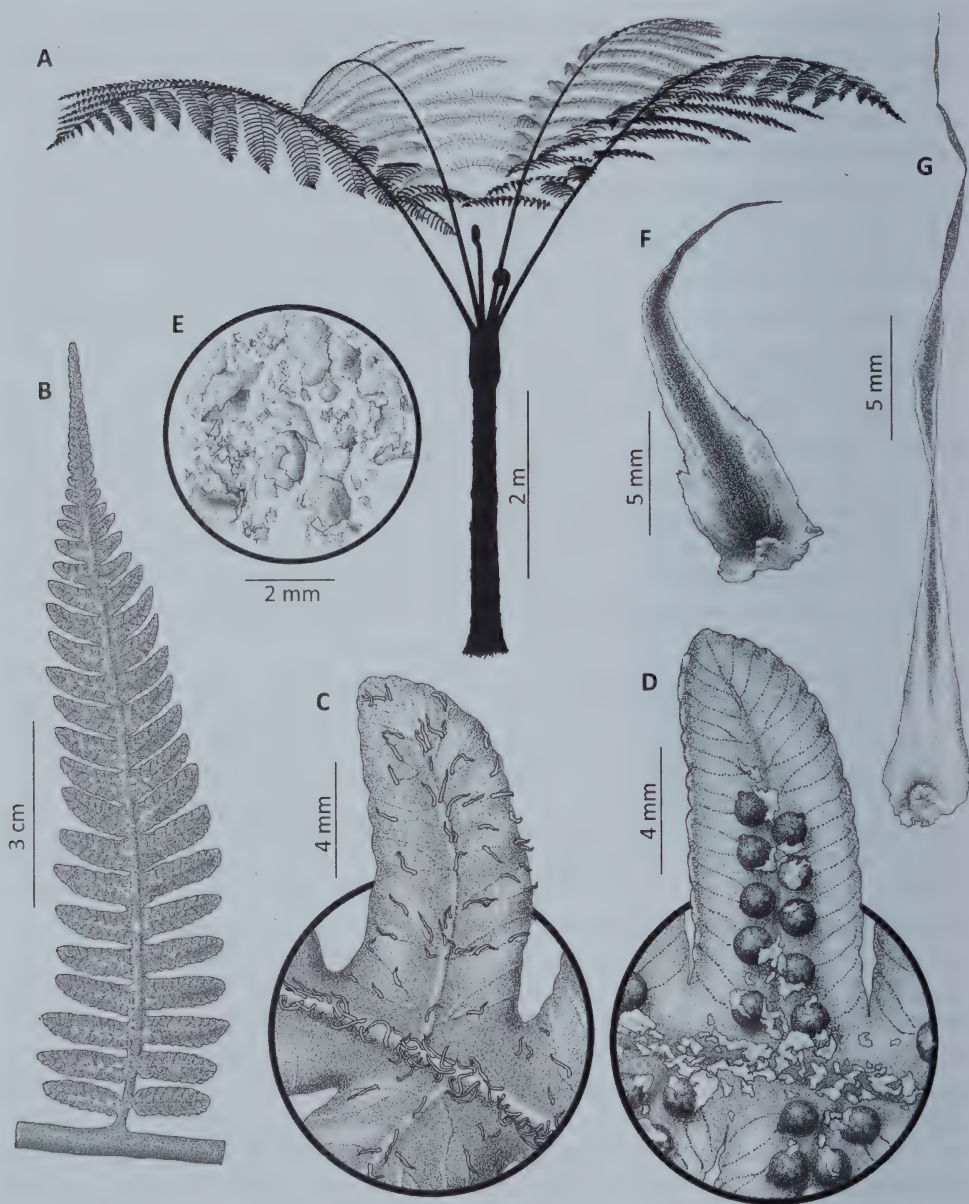


FIG. 7. A-F, *Cyathea monteagudo* (from A. Monteagudo 24166 HOXA). A. habit, silhouette. B. sterile pinnule, abaxially. C. fertile segment, adaxially. D. fertile segment, abaxially. E. petiole scurf squamules. F. petiole scale. G. *Cyathea cystolepis*, petiole scale (from Calatayud. No. USM).

shorter than sporangia. Spores not examined. Hairs and scales: Frond axes adaxially, with sparse indument of brown, thick, tortuous, multicellular hairs, 1.0–2.0 mm long, also present along costae and veins, petioles and rachis abaxially glabrous but with thick, persistent scurf of clumped, ragged cream to

yellowish squamules. Lamina and costae abaxially with abundant cream to yellowish squamules both flat and bullate.

Etymology.—The specific epithet honors Abel Monteagudo, Peruvian botanist and ecologist who has tirelessly collected and preserved tree fern specimens from across the Andes, including the holotype of this new species.

Distribution and habitat.—*Cyathea monteagudoi* is known from two localities in Peru, Chacos in central Peru, and Kosñipata, in southern Peru (Fig. 3), where it grows under tall-canopied cloud forest at 2500–2750 m. In the last locality, it is known only from field photographs. This species was previously misidentified by the authors as *Cyathea cystolepis* Sodiro because of its strongly bicolorous scales, thick, cream to yellowish petiole scurf, and abundant cream to yellowish squamules on the lamina abaxially. The resemblance, however, might be only superficial. *Cyathea monteagudoi*, which may have stronger affinities with the central Andean *Cyathea ruiziana*, differs from *C. cystolepis*, probably more closely related to the *Cyathea straminea* H. Karst. and *Cyathea atahualpa* (R.M. Tryon) Lellinger group, by its long petioles, broadly ovate lamina, pinna and pinnules held mostly horizontally, pinnules short stalked, lanceolate to slightly triangular, segments slightly falcate with acute tips, firm, lanceolate to falcate petiole scales with erose margins, coarse, thick scurf of clumped, ragged squamules on petiole abaxially, and thick, tortuous hairs along costules and veins adaxially (vs. petioles short, narrowly ovate lamina, pinnae twisted, holding proximal pinnules at a high angle, pinnules sessile, narrowly lanceolate, segment tips rounded, papery, narrowly lanceolate petiole scales with entire margins (Fig. 7 G), dense but thin scurf of matted arachnoid squamules, and small, fine, straight hairs along costules and veins in *C. cystolepis*). From its potential close relatives and sympatric species *C. ruiziana* and *C. austropallescens* Lehnert, *C. monteagudoi* can be separated by its smaller stature (trunks to 2.5 m tall), trunk apex tall and narrow in fully grown individuals, ovate fronds, smaller number of fronds (5–7), petiole scurf tan to yellowish, and often falcate petiole scales. *Cyathea ruiziana* and *C. austropallescens* develop tall trunks (larger than 5 m to over 10 m in *C. ruiziana*), have broad trunk apices, longer, elliptical fronds, greater number of fronds (10–15), pale stramineous to grayish white scurf, and straight to apically twisted scales.

Cyathea pibyae A. Tejedor and G. Calatayud, **sp. nov.** TYPE—Peru. Cajamarca: San Ignacio, Huarango, Cordillera Huarango, El Romerillo, - 5.2694, -78.6858, 2370 m, 18 July 2005, *E. Rodriguez* 2893 (holotype: USM!). (Figs. 2J, 8)

Diagnosis.—A small indusiate *Cyathea*, with bipinnate-pinnatisect fronds, similar to *Cyathea gracilis* but differing in its more strongly dissected frond, nearly triangular lamina, concolorous petiole scales, and frequent production of adventitious buds.

Tree fern, trunk to 150 cm tall, erect, slender, to 5 cm diam., often with lateral buds. Fronds to 110 cm long, ascending, with strongly arching apex,



FIG. 8. *Cyathea pibya*. A. habit, silhouette (from photograph by A. Tejedor). B. fertile pinna, silhouette (from E. Rodriguez 2893 USM). C. fertile segment, abaxially. D. sterile pinnule, abaxially. E. petiole scale (from field photograph by A. Tejedor).

lamina to 70×52 cm, broadly ovate, apex acute, gradually reduced, bipinnate-pinnatisect, dark brown adaxially and lighter brown abaxially when dried, bright green in live individuals; frond axes adaxially sericeous, abaxially glabrous, dull brown when dried. Petioles to 40 cm, sparsely verrucate, dark brown when dried; petiole scales 150 mm, covering both adaxial and abaxial sides of basal portion of petiole and only abaxial side to two thirds of distance to basal pinnae, petiole scales lanceolate, concolorous golden-brown, with

margins entire and slightly lighter, tips falcate, long caudate and sinuous to twisted. Pinnae alternate to subopposite, patent to ascending. Longest pinnae 36×12 cm, stalked to 6.2 cm, long ovate to lanceolate, apex acute, costae non-alate. Pinnules to 5.7×1.7 cm, long ovate, tips shortly acute, stalked to 7 mm, with oblique truncate bases; segments to 8×3 mm, oblong, slightly falcate, basal segments remote with margins irregularly crenulate, veins weakly raised abaxially, flat adaxially, fertile veins forked. Sori proximal, to ca. 0.7 mm diam., each with ca. 15 sporangia, shiny brown in dried material, indusia sphaeropteroid, with well-developed umbo, dark brown when dry, paraphyses and spores not examined. Hairs and scales: Frond axes adaxially sericeous, dense indument of brown to translucent multicellular hairs, to 1.0 mm long, matted and appressed; abaxially with thinner similar indument. Costae and costules abaxially with scattered golden translucent to light brown, ovate to lanceolate squamules.

Etymology.—The specific epithet honors Sylvia Gutierrez Valliciergo, affectionately known as Piby among friends and family, mother of the first author and who sparked his fascination with tree ferns by pointing them out in Cuba's countryside.

Paratype.—Peru. Cajamarca: San Ignacio, Huarango, Cordillera Huarango, Camino entre El Romerillo y Nuevo Mundo, -5.2714429, -78.687782, 2322 m, 1 August 2014, G. Calatayud 6033 (CUZ!, USM!)

Distribution and habitat.—*Cyathea pibya*e has been found growing in dense, wet scrub over white sands and sandstone at 2200–2500 m in Huarango, Cajamarca and Abra Patricia, Amazonas-San Martín border (Fig. 3). At Huarango, where it is far more abundant and attains larger sizes, it co-occurs with *Cyathea dudleyi* R.M. Tryon and *Cyathea peladensis* (Hieron.) Domin and replaces *Cyathea chontilla* Lehnert as the dominant tree fern where the sandstone scrub becomes wetter, as one moves from the drier, more open vegetation of the leeward side of mountains into cloud-immersed ridges. In Abra Patricia, it co-occurs with *Cyathea gracilis* Griseb. and *Cyathea oreopteroides* but there it has been found only as stunted, multi-stemmed individuals.

*Cyathea pibya*e is quite distinctive among Neotropical tree ferns in combining a fully arborescent habit and bipinnate-pinnatifid to tripinnate frond with an overall small size. In its general aspect, size, and sphaeropteroid indusium it somewhat resembles *Cyathea ebenina* H. Karst. but can be distinguished from this species by its dull orange-brown, concolorous scales, and its remote proximal segments with crenate margins versus scales bright orange-brown, concordantly bicolorous with dark brown centers, and adjoined proximal segments with entire margins. The holotype of *Cyathea pibya*e was originally identified as *Cyathea lechleri*, probably because of its pinnule dissection and indusium, but the two species differ markedly in their petiole scales (concolorous orange-brown, papyraceous, long lanceolate, and spreading in *C. pibya*e vs. discordantly bicolorous, with dark brown centers and slightly lighter margins, firm, ovate with acute tips, and appressed in *C. lechleri*), petiole scurf (sparse, thin, and easily abraded in *C. pibya*e vs.

relatively thick and persistent in *C. lechleri*), and more importantly in the fronds (broadly ovate to triangular, strongly arching to distally drooping, 2–3 per plant, lax, with 5–7 pinna pairs, basal ones not much shorter than medial ones and patent in *C. pibya* vs. long ovate, patent, 8–10 per plant, compact, with 11–12 pinna pairs, basal ones less than $\frac{1}{2}$ the medial pinna and reflexed in *C. lechleri*).

Cyathea chontilla could be confused with *C. pibya* in the herbarium because of their similar scales and sori, however, the two species differ in the density and distribution of their indument, degree of frond dissection and, especially, in their habits, which are strikingly different in the field. *Cyathea chontilla* is characterized by markedly erect fronds with strongly revolute pinnae and pinnules, and dense crowns with 5–7 fronds. Its axes abaxially are covered by an indument so dense that it obscures the epidermis almost entirely, and formed by thick, matted, tortuous, translucent brown hairs interspersed with scales similar to those of petioles but smaller. Costules and midveins are sparsely hairy with abundant bullate brown squamules. Finally, pinnules of *C. chontilla* are short stalked (stalk length 0.5 the width of basal segments), and basal segments are triangular and adnate. In contrast, *C. pibya* has strongly arching fronds, with drooping tips, and an open crown of only 2–3 fronds, axes abaxially with the epidermis clearly visible through a sparse indument of scattered, appressed, tortuous, opaque brown hairs, costules and midveins glabrous to glabrescent with few bullate brown squamules, long-stalked pinnules (stalk length to 1.5 the width of basal segments), and basal segments oblong and free.

Among Neotropical tree ferns, *Cyathea pibya* is perhaps most similar to *Cyathea gracilis* in its gracefully arching fronds and indusium characters, but differs from this species in its relatively long, slender trunk, with frequent production of lateral buds, and concolorous orange-brown trunk and petiole scales vs. trunk shorter and wider without lateral buds, and scales bicolorous with chestnut-brown centers and light orange-brown margins in *C. gracilis*.

Cyathea recondita A. Tejedor & G. Calatayud **sp. nov.** Type:—PERU. Amazonas: Condorcanqui, Cordillera del Cóndor, cuchilla abajo del Cerro Machinaza, Norte del Puesto de Vigilancia Alfonso Ugarte, cabeceras del río Comainas, 03 53.1S, 78 26.0W, 1750–1800 m, 30 Julio 1994, H. Beltrán 1401 (holotype: USM!). (Fig. 9)

Diagnosis.—An indusiate species of *Cyathea* with bipinnate-pinnatifid laminae that is similar to *Cyathea lasiosora* but differs in having linear, hair-like scales and hemitelioid indusia.

Tree fern, trunk to 3 m tall, erect. Petioles muricate, dull brown when dried; petiole scales covering petiole densely on both adaxial and abaxial side up to half the distance to basal pinnae, narrowly linear, macroscopically almost hair-like to 25×0.5 mm, concolorous orange-brown, margins undifferentiated. Laminae bipinnate-pinnatisect, dark olive-adaxially when dried, lighter brown olive-abaxially, pinnae alternate. Frond axes inermous, dull brown when



FIG. 9. A-G, *Cyathea recondita* (from H. Beltran 1401 USM). A. fertile pinnule, silhouette. B. sorus. C. fertile segment, abaxially, with shed sporangia. D. fertile pinnule, adaxially. E. petiole. F. petiole scale. G. detail of petiole scale. H. *Cyathea traillii*, petiole scale (from field photograph by A. Tejedor).

dried; costae non-alate. Longest pinnae 45×13 cm, stalked to 2 cm. Largest pinnules to 65×18 mm, lanceolate to long triangular, with attenuate tips, stalked, with asymmetric, truncate bases; segments to 15×5 mm, those on the distal half of pinnules often twice as long as those on the proximal half, the margins serrate, veins weakly raised abaxially, flat adaxially, fertile veins forked or simple. Sori medial, to ca. 1 mm diam., each with ca. 10 sporangia, shiny brown in dried material, indusia hemitelioid, ascending, with erose margins, dark brown when dry, paraphyses many, tortuous, as long as sporangia, whitish. Spores pale yellow. Hairs and scales: Frond axes adaxially with dense indument of brown to translucent multicellular hairs, antrorsely curved, 1.0–2.0 mm long, abaxially with whitish tortuous, matted squamules and pale brown bullate squamules.

Etymology.—From the Latin *reconditus*, tucked away, in reference to the inaccessibility of its only known locality, reached by biologists only once after having been dropped on the slopes of Cerro Machinaza by helicopter during a 1994 Field Museum expedition.

Distribution and habitat.—*Cyathea recondita* is currently only known from its type specimen collected at 1750–1800 m on the southern slope of Cerro Machinaza, one of the several middle-elevation sandstone plateaus of the Cordillera del Cóndor (Fig. 3). It was found growing on a steep slope, on a substrate with dense root and moss cover, under a forest with a 15–25m tall canopy, with abundant moss and epiphyte cover on tree trunks.

The most striking morphological feature of *C. recondita* is its extraordinarily dense indument composed of very thin, linear scales that appear to be among the most hair-like of any species in its genus. For this reason, judging from its specimen label, it seems to have been confused with *Lophosoria* C. Presl at the time of collection. *Lophosoria*, a widely distributed Neotropical tree fern genus within the Dicksoniaceae, is characterized by a dense indument of brown to golden or reddish-brown hairs, which covers the trunk and petioles. It differs markedly from *C. recondita* in its fully 3- to 4-pinnate laminae and marginal sori, vs. a bipinnate-pinnatifid lamina and medial sori in *C. recondita*. Probably also due to its peculiar scales, the type specimen of *C. recondita* has also been mistaken for *C. aterrima*, another species with dense, furry orange-brown indument. *Cyathea aterrima*, however, can be readily separated from *C. recondita* by the lack of indusia, the presence of actual white hairs to 8 mm long interspersed with lanceolate orange-brown scales with tortuous tips, and by its hairy lamina, sessile pinnules, and nearly free segments with crenate margins vs. indusium hemitelioid, a glabrous lamina, stalked pinnules, and short segments with dentate margins in *C. recondita*.

The stature and lamina architecture of *Cyathea recondita* probably make this species most similar in habit to *C. calamitatis*, *C. diabolica*, *C. lasiosora*, and *C. traillii*. All these species, which are not necessarily closely related, are slender, medium-sized tree ferns of the forest interior with relatively long-stalked, large pinnules. Besides being easily distinguished by its extremely thin (to 0.5 mm wide), linear scales (scales ovate to lanceolate in *C. calamitatis*, *C. diabolica*, *C. lasiosora*, and *C. traillii*), *C. recondita* has more deeply dissected pinnules,

with thin, oblong segments. The segments of *C. calamitatis*, *C. diabolica*, *C. lasiosora*, and *C. traillii* are triangular and, with the exception of those of *C. traillii*, which have serrated segment margins like those of *C. recondita*, differ in being crenulate (*C. calamitatis* and *C. lasiosora*) or entire (*C. diabolica*). Only one of these species, *C. diabolica*, overlaps in elevation (1600–2400 m) with *C. recondita*, the rest occur below 1300 m.

Cyathea rocioae A. Tejedor & G. Calatayud, **sp. nov.** Type:—PERU. Pasco. Oxapampa: Chacos, base del cerro de la antena, -10.643, -75.29, 2500 m, 13 April 2014, A. Montegudo 25230 (holotype: HOXA!). (Figs. 2K, L, 10.)

Diagnosis.—An indusiate *Cyathea* with bipinnate-pinnatifid laminae that are similar to *C. polliculi* but differ by the dense lanuginose tomentum along axes and surface of laminae adaxially and along axes and veins abaxially, long triangular pinnae, and narrow, pale reddish brown scale margins.

Tree fern, trunk to 20 cm tall, erect, to 7 cm wide. Fronds to 80 cm, patent to arching, slightly drooping at tips. Petioles to 30 cm, sparsely verrucate, dull brown when dried; petiole scales covering petiole to base of lamina, to 10 × 4 mm, getting progressively smaller from petiole base to base of lamina, concordantly bicolorous, reddish brown, with narrow pale reddish brown margins, lanceolate with attenuate, tortuous tips. Laminae to 50 × 30 cm, narrowly triangular, bipinnate-pinnatifid, light olive-brown adaxially and abaxially when dried, pinnae alternate to proximally subopposite. Frond axes inermous, dull brown when dried; costae non-alate. Longest pinnae 16 × 7 cm, stalked to 1 cm, triangular. Largest pinnules to 35 × 10 mm, lanceolate, with rounded tips, sessile, with truncate to slightly cuneate bases; segments to 4 × 2 mm, the margins slightly crenulate and involute, fertile veins simple or rarely forked. Sori proximal, to ca. 1 mm diam., each with ca. 25–30 sporangia, light brown in dried material, indusium hemitelioid, inconspicuous, to 2 mm long, entirely covered by sorus paraphyses many, tortuous, twice as long as sporangia, whitish. Spores not examined. Hairs and scales: Frond axes, veins, and lamina between veins adaxially with dense lanuginose indument of whitish to translucent tortuous hairs, 1 mm long, abaxially with whitish tortuous hairs along axes, and veins, and orange-brown bullate to sub-bullate squamules with lanceolate, tortuous tips, along costules and veins.

Etymology.—The specific epithet honors Rocío Rojas, co-curator of the Oxapampa branch of the Missouri Botanical Garden, who has contributed to building and safeguarding a treasure trove of plants from the Peruvian lowland and montane forests. The collections amassed partly by Rocío, include many plants from Cerro Chacos, the type locality of this species.

Distribution and habitat.—*Cyathea rocioae* is known only from Cerro Chacos near the town of Chacos, Oxapampa, Peru (Fig. 3). The area is notorious for its endemic rich flora growing on white sands and sandstone where many other sandstone specialist tree ferns are found, including *C. chontilla*, *C. serpens* (R.M. Tryon) Lehnert, *C. polliculi* Lehnert, *C. microphylla* Mett., and *C.*

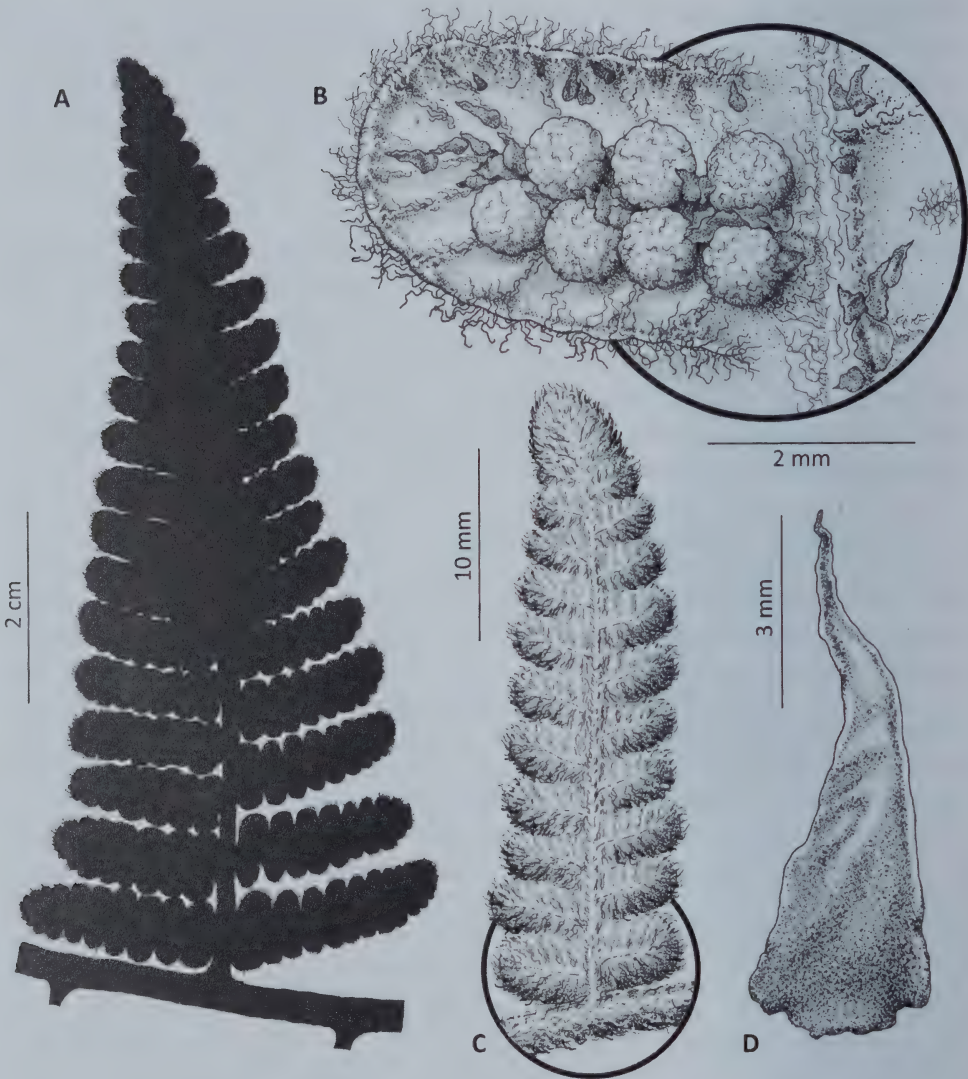


FIG. 10. *Cyathea rocioae*. A. pinna, silhouette. B. fertile segment, abaxially. C. fertile pinnule adaxially. D. petiole scale (from holotype A. Monteagudo 25230 HOXA)

valliciergoana sp. nov. *Cyathea rocioae* is found co-occurring with most of these species on open wet scrub and road cuts.

Cyathea rocioae can be distinguished at a glance from all other congeners by its densely lanuginose lamina abaxially and adaxially. It could be confused with two sympatric species, *Cyathea polliculi* and *C. serpens*. With the former it shares the pinnate-pinnatifid lamina but can be distinguished from it by its light brown instead of whitish petiole scale margins and by the markedly triangular lamina and pinnules (lamina long elliptic and long oblong pinnules

in *C. polliculi*). With *C. serpens* it shares the abundant tortuous hairs (much more dense in *C. rocioae*) but can be distinguished from it by its entire scale margins, and the short to absent pinna and pinnule stalks vs. scale margins fimbriate and pinnae and pinnules long-stalked in *C. serpens*. Two other Andean *Cyathea* that could be confused in herbaria with *C. rocioae* because of their triangular pinnae and hairy lamina are *C. frigida* (H.Karst.) Domin and *C. zongensis* Lehnert. These two species, however, have a broadly triangular, bipinnate-pinnatisect to tripinnate laminae with long, crenulate segments, scales ovate to ovate-lanceolate with acute to obtuse tips, glabrous lamina adaxially between veins, and lack indusia. *Cyathea rocioae*, in contrast, has a narrowly triangular, bipinnate-pinnatifid lamina with short, entire segments, scales lanceolate with attenuate, tortuous tips, a hairy lamina in between veins, and a small, hemitelioid indusium.

Cyathea valliciergoana A. Tejedor & G. Calatayud **sp. nov.** TYPE. Peru. Pasco: Prov. Oxapampa, Chacos, cerro antena, borde de carretera, -10.643, -75.29, 2500–2750 m, 27 March 2014, A. Monteagudo 24687 (holotype: HOXA! isotype: USM!). (Figs. 2M–O, 11)

Diagnosis.—A small indusiate *Cyathea*, with bipinnate-pinnatisect fronds, similar to *Cyathea sylvatica* but differing in its glabrous lamina adaxially, indusium with well-developed umbo, non-alate costa, and abundant bullate squamules in the lamina abaxially.

Tree fern, trunk to 50 cm tall, erect, slender, to 5 cm diam., sometimes with lateral buds. Fronds 60 to 96 cm, ascending, with straight to slightly arched apex, lamina 36 to 62 × 18 to 30 cm, long ovate to triangular, apex acute, gradually reduced, bipinnate-pinnatisect, dark brown adaxially, lighter brown abaxially when dried, bright green in live individuals; frond axes inermous, dull brown when dried. Petioles 24 to 34 cm sparsely verrucate, dark brown when dried; petioles scales covering petiole base on adaxial side with sparse scales extending up to two thirds of the distance to basal pinnae, ovate, nearly concolorous golden-brown with slightly lighter, narrow differentiated erose margins, tips falcate, long caudate and sinuous to twisted. Pinnae alternate, patent to ascending. Longest pinnae 5 × 6 cm, stalked to 8 mm, oblong to lanceolate, apex acute, costae non-alate. Largest pinnules to 2.8 × 0.8 cm, oblong, tips broadly acute, short stalked to 1 mm, with truncate bases; segments to 5 × 3 mm, sinuses narrow along most of pinnule but basal segments free and sometimes remote, margins weakly crenulate, veins weakly raised abaxially, flat adaxially, fertile veins forked. Sori costal, to ca. 0.7 mm diam., each with ca. 10 sporangia, shiny brown in dried material, indusia sphaeropteroid, with well-developed umbo, dark brown when dry, paraphyses few, shorter than sporangia. Spores not examined. Hairs and scales: Frond axes adaxially, with dense indument of brown to translucent multicellular hairs, matted, and appressed, 1.0–2.0 mm long, and scattered linear-lanceolate squamules, abaxially glabrous but with thin ephemeral scurf of golden brown

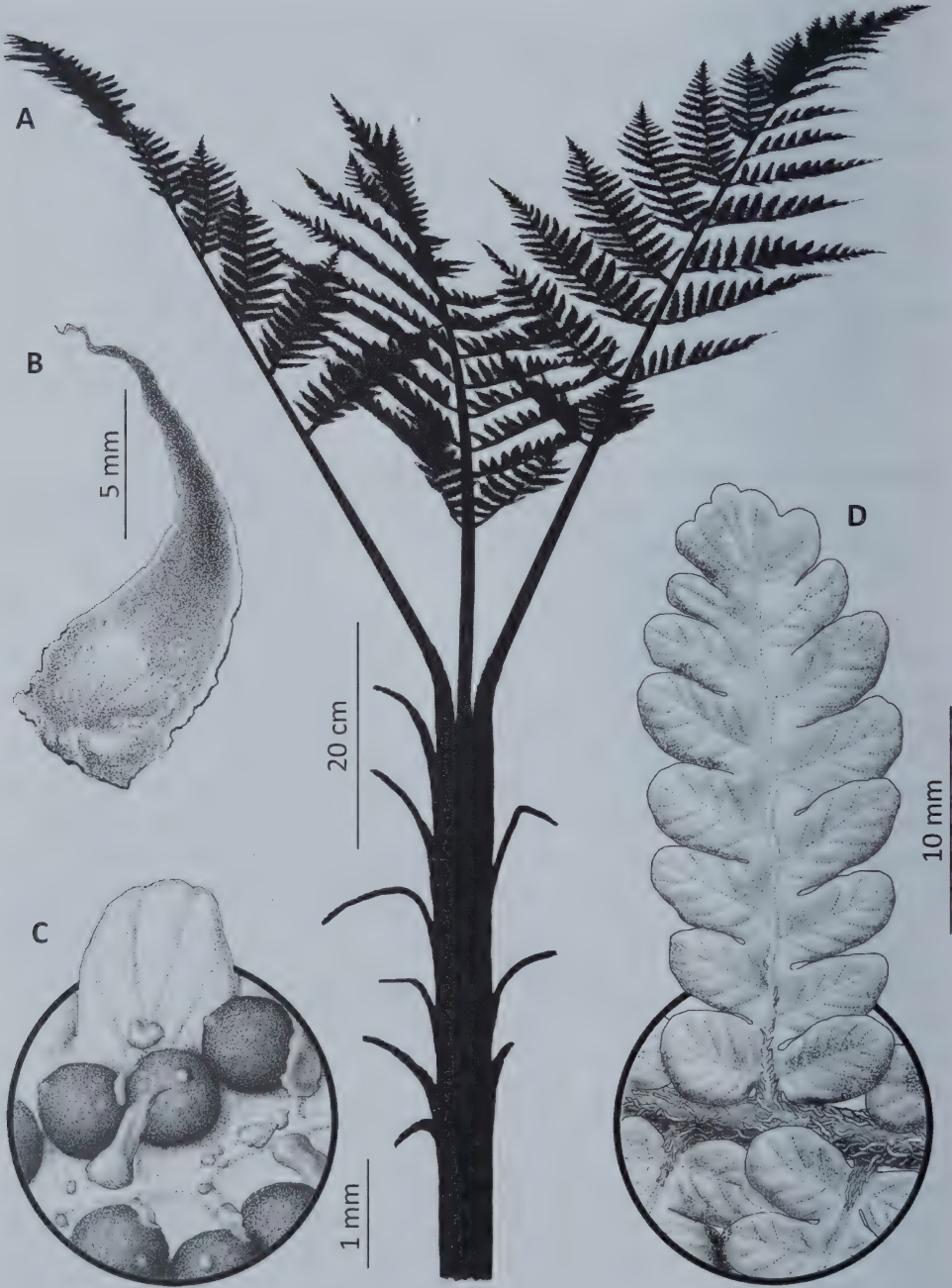


FIG. 11. *Cyathea valliciergoana* (from holotype A. Monteagudo 24687 HOXA). A. habit, silhouette. B. petiole scale. C. Fertile segment, abaxially. D. fertile pinnule, adaxially.

arachnoid squamules. Lamina and costae abaxially with abundant but scattered golden brown squamules both flat and bullate.

Etymology.—Named after Sylvia Valliciergo Barrena, grandmother of AT and unfailing supporter of his fondness for wild places and creatures.

Distribution and habitat.—*Cyathea valliciergoana* is known thus far from a single locality, Cerro Chacos, at 2500–2750 m, near Oxapampa, Pasco, Peru (Fig. 3). There it grows in fairly large numbers in perhumid, open scrub vegetation over white sandstone and white sand soils. Its relatively stiff, ascending fronds could be an adaptation to water conservation in such an exposed habitat, where it sporadically may have to cope with short periods of intense solar radiation and quick draining soils.

Cyathea valliciergoana may be confused with *C. sylvatica* Lehnert known thus far only from elfin forest vegetation near Vilcabamba, southern Ecuador, also at 2600 m (Lehnert 2006). The two species are extraordinarily similar in their small size and ascending fronds with long petioles, but *C. sylvatica* differs in its abundant tortuous hairs along veins adaxially, narrowly green alate costae, crenate to pinnatifid pinnules, indusium without an umbo, and lack of bullate squamules on the lamina abaxially. In *C. valliciergoana*, the lamina is glabrous adaxially, costae are non-alate, pinnules are pinnatisect to basally pinnate, with free and remote segments, the indusium has a well-developed umbo, and the lamina abaxially has abundant golden-brown flat and bullate squamules. The two species could in fact not be closely related. Lehnert (2006) suggests that *C. sylvatica* may be closest to *C. patens*, whereas *C. valliciergoana* appears to have a stronger affinity to the sympatric *C. dudleyi*.

With *Cyathea dudleyi*, *C. valliciergoana* shares an erect, slender trunk that has a tendency to produce lateral buds, a long ovate to triangular lamina, free, remote basal pinnule segments, indusium with well-developed umbo, and abundant bullate squamules on the lamina abaxially. From a distance, *C. valliciergoana* can be readily separated from *C. dudleyi* by its overall smaller size (trunks < 1 m tall in *C. valliciergoana* and attaining up to 2.5 m in *C. dudleyi*) and by its ascending, stiff fronds and pinnae, that sometimes arch only slightly at the tips (fronds and pinnae broadly arching to slightly drooping at tips in *C. dudleyi*). On closer inspection, *C. valliciergoana* differs from *C. dudleyi* in its more weakly divided lamina (most segments adnate, separated by narrow sinuses that do not reach costules in *C. valliciergoana* vs. most segments free and remote in *C. dudleyi*) and in its well-developed indument (dense cover of matted, appressed hairs along axes adaxially and thin scurf of arachnoid squamules abaxially vs. axes mostly lacking indument in *C. dudleyi*).

The characters that separate *C. valliciergoana* from *C. dudleyi* suggest adaptations to open, low scrub vegetation in the former. These differences are consistent even when the two species grow together in exposed locations, where *C. dudleyi*, albeit severely stunted, retains its strongly arching fronds, nearly tripinnate lamina, and clean, scurfless axes. These potential adaptations to an intermittently sun-drenched habitat in *C. valliciergoana* (i.e., ascending

fronds and pinnae plus dense indument) are taken to a further extreme in the also sympatric *C. chontilla*, in which both fronds and pinnae are not only ascending but markedly revolute, and have a dense scale cover along axes abaxially. The strong habit resemblance between *C. valliciergoana* and *C. chontilla* is such that the two species can be easily confused with each other at a distance. At closer range, however, *C. valliciergoana* can be readily separated from *C. chontilla* by its straight to slightly arching frond and pinna apices, pinnae narrowly oblong lanceolate, pinnae alternate and imbricate, petiole scales ovate and flattish, mostly confined to petiole base, basal pinnule segments free and remote, and brighter green, softer lamina vs. frond and pinnae revolute, pinna broadly oblong to triangular, subopposite and often remote, scales long lanceolate and strongly twisted, extending along entire petiole and rachis, pinnule segments adnate, and lamina dark green and chartaceous in *C. chontilla*. These differences between the two species are consistent in both juvenile and adult stages (Fig. 2). In many traits, *C. valliciergoana* appears to be intermediate between *C. chontilla* and *C. dudleyi*, and it is not clear whether it is the result of hybridization between the last two species or if it is just a close relative of *C. dudleyi* that is convergent with *C. chontilla* because of the exposed habitat shared by the two species. Even if it is a hybrid, it is represented by a large, multi-generation population that stretches across several kilometers and should thus be regarded as a fully distinct species.

Cyathea xerica A. Tejedor & Calatayud, **sp. nov.** Type: Peru. Amazonas: Corosha, Copal, camino a la reserva Hierbabuena-Allpayacu, -5.7927, -77.83, 2430 m, 24 July 2014, G. Calatayud 5621 (holotype: USM!, isotype CUZ!). (Figs. 2P, 12)

Diagnosis.—An indusiate species of *Cyathea* with bipinnate-pinnatifid lamina that is similar to *Cyathea chontilla* but differs in its matted hair tomentum on the lamina adaxially, its drooping pinnules and pinnae tips, and creeping rhizome.

Rhizome creeping, 15–20 cm long, 3 cm diam., covered with old petiole bases, with adventitious buds. Fronds to 78–80 cm long, erect, strongly ascending with drooping apex. Petioles 27–28 cm long, sparsely verrucate, pale olive-green to atropurpureous basally. Petiole scales lanceolate to long lanceolate, 10–16 × 2–2.5 mm, concolorous translucent brown, with irregular margins and slightly sinuous long acute tips, scattered on adaxial side, extending along petiole to half the distance to basal pinnae. Lamina to 76 × 25 cm, elliptic, bipinnate – pinnatisect, herbaceous, pale grayish green in live individuals, apex acute, gradually reduced. Rachis, brown. Pinnae to 14 × 4.5 cm long, stalked to 6 mm, patent, alternate to subopposite, distal segments simply adnate before ending in a pinnatifid apical section; medial pinnae larger than the basal pair. Costa 1 mm wide, olive-green to dark brown in fresh material adaxially and abaxially, non-alate. Largest pinnules 1.7–2.2 × 7–10 mm, stalked to 1–3 mm, ovate, truncate basally, acute apically; costules olive-



FIG. 12. *Cyathea xerica* (from holotype 5621 G. Calatayud USM). A. habit. B. petiole scale. C. fertile segment, abaxially.

green adaxially and abaxially, brown in dry material. Segments to 6.0×2.0 mm, sessile, adnate, patent to slightly ascending, tips obtuse, proximal segments subopposite basally, longer than following segments; sinuses acute, to 0.2 mm wide; segment margins slightly crenulate, markedly involute; veins planar on both sides, olive-green and dark brown in dry material, sterile and fertile veins simple. Sori 1.0 mm diam., costal, rich orange-brown when mature; indusia sphaeropteroid; spores pale brown. Hairs and scales: Rachis adaxially with abundant pale stramineous multicellular hairs; costa densely pubescent, with multicellular long hairs tan to white 1.0–1.5 mm long,

adaxially and abaxially; costules densely hairy, with brown translucent, multicellular hairs to 1.0 mm long, adaxially; abaxially with many tan squamules, sub-bullate to flat 0.8–1.0 mm long, flat lanceolate ones to 3×2 mm, and white hair-like linear tips, veins adaxially with densely white multicellular hairs to 0.3 mm long on the midveins, abaxially midveins with light brown squamules, mostly bullate ones to 0.2 mm, and scattered light brown squamules to 1.5 mm long.

Etymology.—The epithet is a Latinized form of *xiros*, Greek for dry, after the dry, scantily vegetated habitats where the species is commonly found.

Paratype.—Peru. Amazonas: Chachapoyas, Molinopampa, Ocol, -6.238375, -77.596308, 2507 m, 12 July 2014, *G. Calatayud* 5975 (USM!).

Distribution and habitat.—*Cyathea xerica* has been found at the northern and southern extremes of a 70 km long chain of sandstone outcrops that spans the southwestern flanks of Cerro Campanario, Amazonas, between 2300–2800 m (Fig. 3). It grows exclusively under full sun on very open scrub and grassland dominated by Eriocaulaceae, sometimes on nearly bare sands or among rocks that support hardly any other vegetation at all. It may be the tree fern in the Andes best adapted to xeric conditions. It is, however, the sand's infertility and quick drainage rather than climate that leads to the low biomass of *C. xerica*'s environment, as the vegetation patches where it is found are surrounded by lush cloud forest wherever the sandstone bedrock is replaced by limestone.

Because of its ascending fronds, *Cyathea xerica* in the field may appear deceptively similar to *Cyathea chontilla*, which occupies similar environments but has not been found co-occurring with *C. xerica*. *Cyathea chontilla*, however, is characterized by stiffly ascending pinnae and pinnules, rather than drooping ones as in *C. xerica*. Both morphologies, albeit divergent, are probably similarly effective adaptations to minimizing exposure to the midday sun during dry days. An additional difference of *C. xerica* is its creeping trunk, erect in *C. chontilla*, which may be a further adaption in the former to maximizing water uptake and conservation in an intermittently desiccating environment. In dry material, the two species may also be confused because both share oblong to triangular pinnules with abundant lanceolate scales and bullate squamules abaxially, as well as sori with sphaeropteroid indusia. In this context, *Cyathea xerica* can be distinguished by its laminar indument of matted whitish hairs adaxially vs. lamina glabrous adaxially in *C. chontilla*.

Cyathea yambrasensis A. Tejedor & G. Calatayud, **sp. nov.** TYPE: Peru. Amazonas: Bongará, Yambrasbamba, Alto Nieva. -5.664, -77.777, 1890 m, date , *G. Calatayud* 5615 (holotype: USM! CUZ!). (Figs. 2Q, R, 13)

A small indusiate *Cyathea* with bipinnate-pinnatisect fronds, similar to *Cyathea gracilis* but different in its acute pinnule apex, short stalked pinnules, non-drooping pinna apices, and sub-bullate squamules on the lamina abaxially.

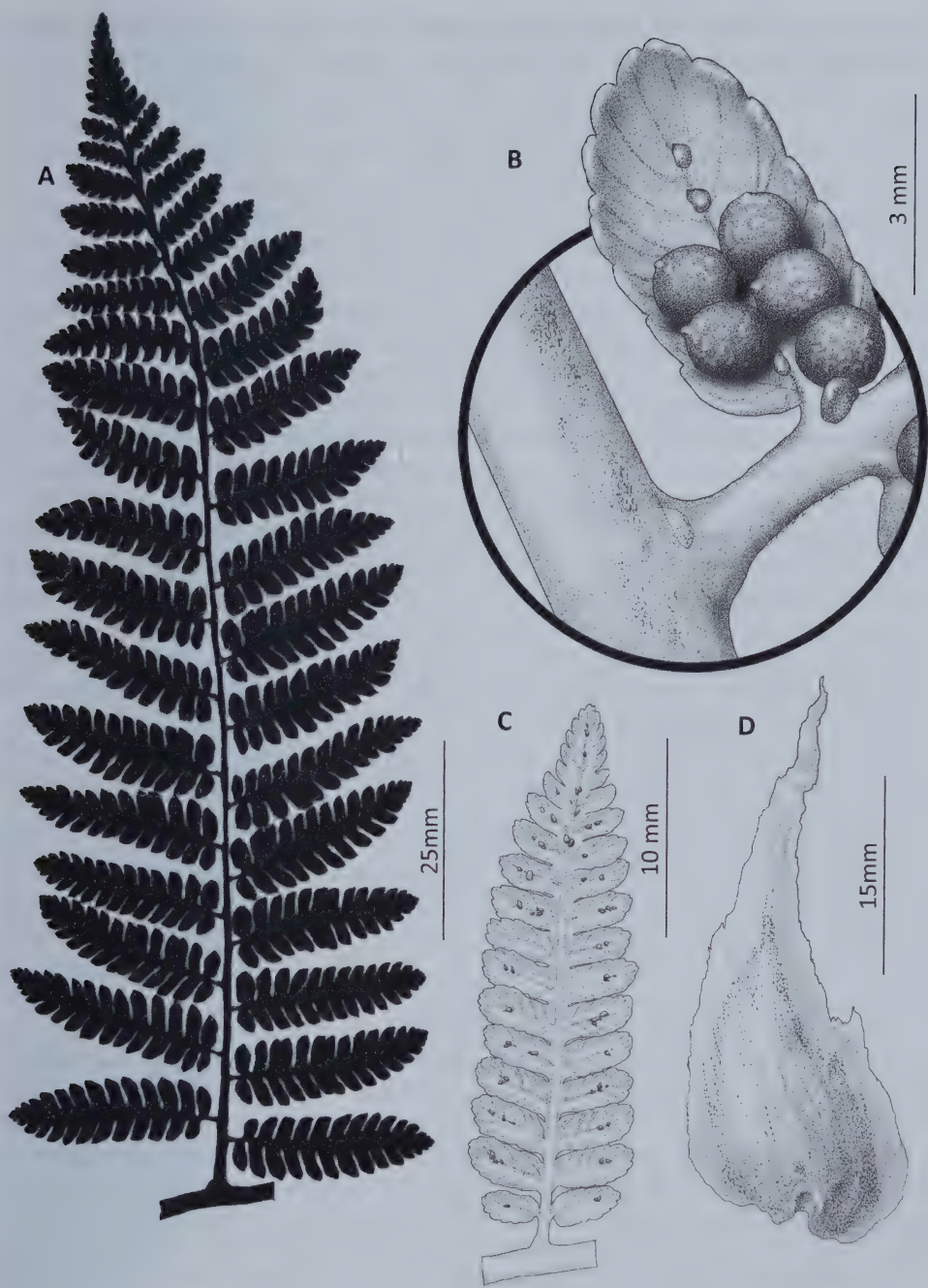


FIG. 13. *Cyathea yambrasensis* (from holotype G. Calatayud 5615 USM). A. fertile pinna, silhouette. B. fertile segment, abaxially. C. sterile pinnule, abaxially. D. petiole scale.

Tree fern, trunk to 40 cm tall, erect, with old petiole bases, to 5 cm diam.; adventitious buds absent. Fronds to 110 cm long, tightly arranged around apex, patent, slightly arching. Petioles to 30 cm long, sparsely verrucate, green or reddish brown when older, with an interrupted line of elongate pneumathodes on each side, to 15×2 mm, white or brown when older; petioles scales only on petiole bases, ovate-lanceolate, to 16×4 mm, orange-brown, with darker, translucent margins, not sharply contrasted with lighter, opaque centers, margins fragile and erose, apices caudate, sinuous to twisted. Laminae to 80×60 cm, ovate, bipinnate-pinnatisect to tripinnate, bright green adaxially (olive-when dried), paler grayish green abaxially, with 9–10 pinna pairs, alternate, basal ones not or weakly reflexed, shorter than longest central pinnae. Frond axes inermous, yellowish green to orange or reddish brown; costae basally with a small elliptic pneumathode, ca. 2×1 mm, yellowish brown, weakly raised, costae narrowly green-alate between the pinnules in distal fourth. Longest pinnae 35×13 cm, lanceolate, long-stalked to 3 cm. Largest pinnules to 60×13 mm, lanceolate with acute tips, stalked to 5 mm, nearly fully pinnate in lower third, basal segments free; segments to 8×3 mm, the margins crenate, veins flat, fertile veins forked. Sori proximal, to ca. 1.1 mm diam., each with ca. 30–40 sporangia, light purplish brown in fresh material, indusia sphaeropteroid, with well-developed umbo, fragile and translucent grayish receptacles and paraphyses not observed. Spores pale yellow. Hairs and scales: Frond axes adaxially and abaxially glabrous, orange-brown bullate squamules abaxially frequent on midveins.

Etymology.—The specific epithet refers to the Peruvian district Yambrasbamba, shortened by locals to Yambras, which harbors the type locality of the species.

Distribution and habitat.—*Cyathea yambrasensis* has been found in two neighboring localities of the Abra Patricia area near the border between Amazonas and San Martín in northern Peru (Fig. 3). It has been found growing in scrubby forest atop sandstone ridges, underneath the partial shade of shrubs.

Because of its coriaceous texture, short stature, and strongly dissected pinnules, we first confused it with *Cyathea chontilla*, which grows in similar environments although in more exposed habitats. It however, differs from this species in the glabrous axes abaxially, indusium lacking an umbo, oblanceolate pinnules, and broad, ovate scales with strongly erose margins vs. axes hairy abaxially, indusium with an umbo, triangular pinnules, and long lanceolate scales with entire margins. The two species are also markedly different in habit. *Cyathea yambrasensis* has patent to slightly arching fronds, with pinnae and pinnules in one plane, whereas *C. chontilla* has stiffly ascending fronds, pinnae and pinnules. Because of its lamina division, tough texture, stalked pinna and pinnules, and near lack of indument on lamina and axes *C. yambrasensis*, however, appears to be a closer relative of *Cyathea gracilis*. It differs from that species in the more compact, flatter frond that arches only slightly, in the reduced basal segments, acuminate pinnule apex, petiole scales with lighter centers, and bullate squamules along midveins abaxially vs. fronds lax, markedly arching, with drooping apices, basal

segments as large or larger than middle segments, attenuate to caudate pinnule apex, petiole scales with dark brown centers and lighter brown margins, and no bullate scales on the lamina abaxially.

DISCUSSION

The species described here increase the known diversity of tree ferns of Peru by nearly 10% relative to the most recent lists (Lehnert, 2011; Tejedor and Calatayud, 2017), bringing the country's total to 101 tree fern species. Most of these new species were found in the Huancabamba Gap zone of northern Peru, and in the Yanachaga cordillera of central Peru, both notoriously rich in ferns and endemic plants in general (Ayers, 1999; Gouda and Manzanares, 2008; Knapp, 2010; Neill, 2005; Schulenberg and Weigend, 2002; Silva and Correa, 2002; Smith 2006; Ulmer and Schwerdtfeger, 2000). Both the Huancabamba Gap zone and the Yanachaga cordillera have particularly diverse geologies that include alternating strata of limestone and sandstone (Campbell, 1974; Duellman and Lehr, 2009), which create an edaphic and geomorphologic mosaic of ridges and plateaus isolated by deep gorges that may contribute to the high species richness of these regions (Knapp, 2002; Weigend, 2002, 2004). The species described here enlarge the lists for these areas and emphasize the role that soil heterogeneity and, in particular, sandstone outcrops have in increasing local plant diversity along the eastern slopes of the Andes. The sandstone outcrops support highly endemic floras that stand in sharp contrast to those occurring in surrounding rocks and often include lineages that are shared with floras growing on the more ancient sandstones, quartzites, and granites of the Guiana Shield (Neill, 2005). As it is typical of vegetation growing on quartz sands and sandstones across the wet tropics (Duivenvoorden and Cleff, 1994; Fine and Bruna, 2016; Miyamoto, 2007; Takeuchi, 1960), Andean sandstone outcrops are often covered by stunted vegetation, forming, in the most exposed locations, scrub and even grasslands of various degrees of density rather than forest.

Except for *Cyathea lehnertii* and *C. monteagudo*, which belong to mostly Andean species groups, the majority of the ferns described here show connections with the Guiana Shield. *Cyathea estevesorum* seems to have affinities with the *Cyathea macrosora* group (including also *C. traillii*, *C. rufescens*, *C. vaupensis*, and *C. macrocarpa* (C. Presl) Domin) which has a wide distribution on sandstone or sandy soils at low elevations from southern Bahia, Brazil, across the Guiana shield, and into the white sands and sandstones of the northwestern Amazon and Andean foothills (Weigand and Lehnert, 2016). *Cyathea estevesorum* may represent an incursion of the *C. macrosora* group into higher elevations in the Andes. Similarly, *C. lechleri*, a possible close relative of *C. pibya* and of the possible members of the *C. chontilla* alliance described here (including *C. valliciergoana* and *C. xerica*), also occurs both in Andean and Guiana sandstones at lower middle elevations. As is the case for the Andean species related to *C. macrosora*, these possible

relatives of *C. lechleri* all occur at higher elevations (greater than 2000 m) than their more widespread relative.

Four of the species described here (*Cyathea estevesorum*, *C. lehnertii*, *C. monteagudoii*, and *C. recondita*) are medium to large-sized plants of the forest interior and five (*C. angelica*, *C. pibya*, *C. rocioae*, *C. valliciergoana*, and *C. xerica*) are plants of small stature that grow in dense to open scrub or among herbaceous vegetation. These small species, despite occurring in areas of consistent high rainfall throughout the year, show traits that are probable adaptations for water conservation on quick-draining sandy soils during short periods of intense water stress, such as cloudless, sunny days. Water conservation traits may include pinnules bent at high angles that probably minimize exposure to the midday sun. This is achieved either by markedly ascending fronds (e.g., *C. valliciergoana*, *C. xerica*) and/or by markedly drooping pinnules (*C. xerica*, and *C. pibya*). Hairy axes (in *C. angelica*, *C. valliciergoana*, and *C. xerica*) and/or lamina (in *C. angelica*, *C. rocioae*, and *C. xerica*) may also contribute to water conservation in the exposed habitats where these species occur.

So far, only one of the newly described species, the non-sandstone restricted *Cyathea monteagudoii*, has been found to occur over a relatively large range (about 650 km long) extending from central to southern Peru. The rest appear to be highly localized geographically, being known in the most extreme cases from a single mountain (two species in Cerro Chacos) or a single cliff (*C. angelica* in Colán). However, hundreds of hard to access and potentially tree fern rich sandstone ridges and plateaus, scattered from the eastern slopes of the Colombian to the central Peruvian Andes, remain poorly explored. It is likely that with additional surveys of patches of perhumid montane scrub, the ranges of some of the species described here may be found to be more extensive, albeit possibly disjunct. This has been the case for *C. chontilla* and *C. polliculi*, each of which is known from only two small populations 750, and 950 km apart, respectively. Moreover, given the rate of description of new tree fern taxa from sandstone scrub in recent years and the small proportion of this habitat sampled so far, it is highly likely that several additional species remain undiscovered across the geologic mosaic of the eastern equatorial Andes. We predict that a high proportion of any new tree ferns described in the future from the region will be species of small stature, both because they tend to dominate the so far prolific sandstone scrubs and because, even in relatively well-sampled areas, they tend to be overlooked in botanic collection efforts that do not focus on ferns.

The reasons why scrub vegetation over sandstone is so fruitful an environment for species of *Cyathea* remain obscure (Lehnert and Tejedor, 2016). One possibility is that *Cyathea* conserves an ancestral niche that favors gametophyte germination in acidic soils (Marcon et al., 2017, Medeiros et al., 2017, Rechenmacher, Schmitt, and Droste 2010) under medium light intensity (Hiendlmeyer and Randi, 2007), which in areas of high precipitation stability close to equatorial latitudes, may lead to larger populations of adults and lower extinction rates. Also, large mixed-species gametophyte carpets may increase opportunities for hybridization and speciation through polyploidy (Barring-

ton, Haufler, and Werth, 1989) or allohomoploidy (Conant and Cooper-Driver, 1980), accelerating diversification rates within sandstone-dwelling lineages. Future phylogenetic and ecological studies of *Cyathea* will provide opportunities for testing these hypotheses, and should help shed light on the mechanisms driving species diversity and community assembly among Andean tree ferns.

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